



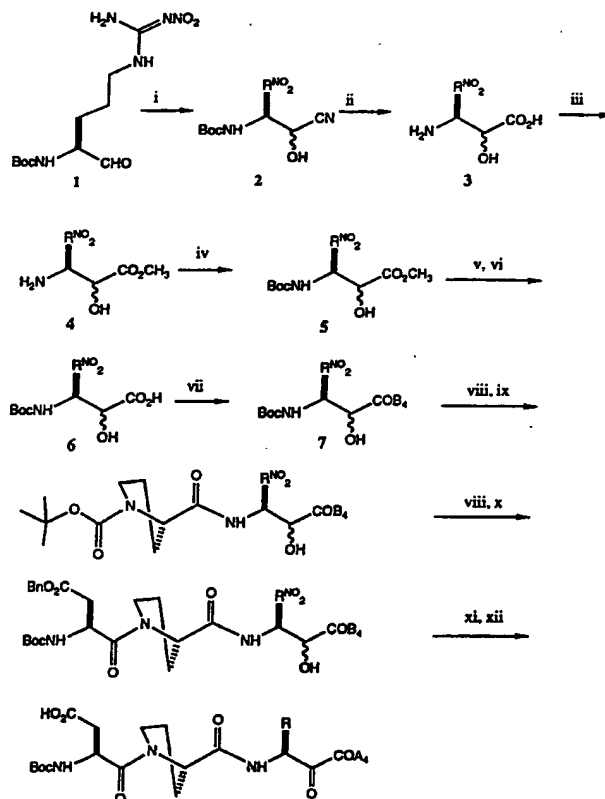
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07C 69/76, 303/00, 233/00 A61K 37/00	A1	(11) International Publication Number: WO 94/08941 (43) International Publication Date: 28 April 1994 (28.04.94)
(21) International Application Number: PCT/US93/10015 (22) International Filing Date: 18 October 1993 (18.10.93) (30) Priority data: 07/962,301 16 October 1992 (16.10.92) US (71) Applicant: CORVAS INTERNATIONAL, INC. [US/US]; 3030 Science Park Road, San Diego, CA 92121 (US). (72) Inventors: WEBB, Thomas, Roy ; 2250 Colony Terrace, Encinitas, CA 92024 (US). MILLER, Todd, Anthony ; 1710 South El Camino Real, #E-208, Encinitas, CA 92024 (US). VLASUK, George, Phillip ; 3024 Garboso Street, Carlsbad, CA 92009 (US). ABELMAN, Matthew, Mark ; 873 Stevens Avenue, #3312, Solana Beach, CA 92075 (US).		(74) Agents: BIGGS, Suzanne, L. et al.; Lyon & Lyon, 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US). (81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>

(54) Title: ARGININE KETO-AMIDE ENZYME INHIBITORS

(57) Abstract

The present invention is directed to novel arginine alpha-keto-amide derivatives, their pharmaceutically acceptable salts and compositions thereof which are useful as antithrombotic agents in mammals and also the use of these compounds as antithrombotic agents. Also, described are methods of using these inhibitors as inhibitors of coagulation proteases and as therapeutic agents for disease states characterized by abnormal thrombus formation and/or disorders of the blood coagulation process. Further described herein are compounds useful as intermediates in the preparation of these compounds.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

DESCRIPTIONArginine Keto-Amide Enzyme InhibitorsRelated Applications

5 This application is a continuation-in-part of U.S. Serial No. 07/962,301, filed October 16, 1992, entitled "Arginine Keto-Amide Enzyme Inhibitors", pending in the Patent Office and hereby incorporated by reference herein, including the drawings attached thereto.

10

Field of the Invention

 The present invention relates in one aspect to novel compounds, their pharmaceutically acceptable salts and pharmaceutically acceptable compositions thereof
15 which are useful as potent and specific inhibitors of blood coagulation in mammals. In another aspect, the invention relates to a methods of using certain of these inhibitors as therapeutic agents for disease states characterized by disorders of the blood coagulation
20 process. In yet another aspect, the invention relates to intermediate compounds for the preparation of the inhibitors.

Background and Introduction to the Invention

25 Normal hemostasis is the result of a complex balance between the processes of clot formation (blood coagulation) and clot dissolution (fibrinolysis). The complex interactions between blood cells, specific plasma proteins and the vascular surface, maintain the
30 fluidity of blood unless injury and blood loss occur.

 Blood coagulation is the culmination of a series of amplified reactions in which several specific zymogens of serine proteases in plasma are activated by limited proteolysis. Nemerson, Y. and Nossel, H.L., Ann. Rev.
35 Med., 33: 479 (1982). This series of reactions results in the formation of an insoluble fibrin matrix which is required for the stabilization of the primary hemostatic

plug. The interaction and propagation of the activation reactions occurs through the extrinsic and intrinsic pathways of coagulation. These pathways are highly interdependent and converge in the formation of the serine protease, Factor Xa. Factor Xa catalyzes the penultimate step in the blood coagulation cascade which is the formation of the serine protease thrombin. This step occurs following the assembly of the prothrombinase complex which is composed of factor Xa, the non-enzymatic co-factor Va and the substrate prothrombin assembled on the surface of adhered, activated platelets or systemically circulating membranous microparticles.

Proteolytic activation of zymogen factor X to its catalytically active form, factor Xa, can occur by either the intrinsic or extrinsic coagulation pathways. The intrinsic pathway is referred to as "intrinsic" because everything needed for clotting is in the blood. Saito, H., "Normal Hemostatic Mechanisms", Disorders of Hemostasis, pp. 27-29, Grune & Stratton, Inc. (O. D. Ratnoff, M.D. and C. D. Forbes, M.D. edit. 1984). This pathway is comprised of the zymogen serine proteases, factors IX and XI, and the non-enzymatic co-factor, factor VIII. The initiation of the intrinsic pathway results in the activation of factor XI to XIa. Factor XIa catalyzes the activation of factor IX to factor IXa which in combination with the activated form of factor VIII on an appropriate phospholipid surface, results in the formation of the tenase complex. This complex also catalyzes the formation of the serine protease, factor Xa, from its zymogen, factor X which subsequently results in clot formation.

The extrinsic pathway is referred to as "extrinsic" because the tissue factor which binds to and facilitates the activation of factor VII comes from outside the blood. Saito, Id. The major components of this pathway are the zymogen serine protease, factor VII, and the membrane bound protein, tissue factor. The latter

serves as the requisite non-enzymatic co-factor for this enzyme. The initiation of this pathway is thought to be an autocatalytic event resulting from the activation of zymogen factor VII by trace levels of activated factor VII (factor VIIa), both of which are bound to newly exposed tissue factor on membrane surfaces at sites of vascular damage. The factor VIIa/tissue factor complex directly catalyzes the formation of the serine protease, factor Xa, from its zymogen, factor X. Exposure of blood to injured tissue initiates blood clotting by the extrinsic pathway.

Proteolytic activation of zymogen factor X to its catalytically active form, factor Xa, results in the liberation of a 52 amino acid activation peptide from the amino-terminus of the heavy chain subunit. The intrinsic activation reaction is catalyzed by factor IXa in a macromolecular complex with the non-enzymatic co-factor, factor VIIIa. Factor Xa formation via the extrinsic pathway is catalyzed by the catalytic complex of factor VIIa and tissue factor. Both of these reactions must occur on an appropriate phospholipid surface in the presence of calcium ions. The active product formed following either intrinsic or extrinsic activation of factor X is α -factor Xa. A second proteolytic cleavage which is thought to be autocatalytic, results in the formation of β -factor Xa following the release of a 14 amino acid peptide from the carboxy-terminus of the heavy chain. Both forms of the activated molecule have the same catalytic activity as measured by their ability to promote coagulation in plasma or hydrolyze a peptidyl chromogenic substrate.

The formation of thrombin is catalyzed by factor Xa following the assembly of the catalytic prothrombinase complex as reviewed by Mann, K. G. et. al., "Surface-Dependent Reactions of the Vitamin K-Dependent Enzyme Complexes", Blood, 76: 1-16 (1990). This complex is composed of factor Xa, the non-enzymatic co-factor Va

and the substrate prothrombin all assembled on an appropriate phospholipid surface. The requirement of a macromolecular complex for efficient catalysis results in the protection of factor Xa from natural anticoagulant mechanisms such as heparin-antithrombin III mediated inhibition. Teite, J. M. and Rosenberg, R. D., "Protection of Factor Xa from neutralization by the heparin-antithrombin complex", J. Clin. Invest., 71: 1383-1391 (1983). In addition, sequestration of factor Xa in the prothrombinase complex also renders it resistant to inhibition by exogenous heparin therapy which also requires antithrombin III to elicit its anticoagulant effect.

Thrombin is the primary mediator of thrombus formation. Thrombin acts directly to cause formation of insoluble fibrin from circulating fibrinogen. In addition, thrombin activates the zymogen factor XIII to the active transglutaminase factor XIIIa which acts to covalently stabilize the growing thrombus by crosslinking the fibrin strands. Lorand, L. and Konishi, K., Arch. Biochem. Biophys., 105: 58 (1964). Beyond its direct role in the formation and stabilization of fibrin rich clots, the enzyme has been reported to have profound bioregulatory effects on a number of cellular components within the vasculature and blood. Shuman, M.A., Ann. NY Acad. Sci., 405: 349 (1986).

It is believed that thrombin is the most potent agonist of platelet activation, and it has been demonstrated to be the primary pathophysiologic-mediator of platelet-dependent arterial thrombus formation. Edit, J.F. et al., J. Clin. Invest., 84: 18 (1989). Thrombin-mediated platelet activation leads to ligand-induced inter-platelet aggregation principally due to the bivalent interactions between adhesive ligands such as fibrinogen and fibronectin with platelet integrin receptors such as glycoprotein IIb/IIIa which assume their active conformation following thrombin activation.

Berndt, M.C. and Phillips, D.R., Platelets in Biology and Pathology, pp 43-74, Elsevier/North Holland Biomedical Press (Gordon, J.L. edit. 1981). Thrombin-activated platelets can also support further thrombin production through the assembly of new prothrombinase and tenase (factor IXa, factor VIIIa and factor X) catalytic complexes on the membrane surface of intact activated platelets and platelet-derived microparticles, following thrombin-mediated activation of the non-enzymatic cofactors V and VIII, respectively. Tans, G. et al., Blood, 77: 2641 (1991). This positive feedback process results in the local generation of large concentrations of thrombin within the vicinity of the thrombus which supports further thrombus growth and extension. Mann, K.G. et al., Blood, 76: 1 (1990).

In contrast to its prothrombotic effects, thrombin has been shown to influence other aspects of hemostasis. These include its effect as an important physiological anticoagulant. The anticoagulant effect of thrombin is expressed following binding of thrombin to the endothelial cell membrane glycoprotein, thrombomodulin. This is thought to result in an alteration of the substrate specificity of thrombin thereby allowing it to recognize and proteolytically activate circulating protein C to give activated protein C (aPC). Musci, G. et al., Biochemistry, 27: 769 (1988). aPC is a serine protease which selectively inactivates the non-enzymatic cofactors Va and VIIIa resulting in a down-regulation of thrombin formation by the prothrombinase and tenase catalytic complexes, respectively. Esmon, C.T., Science, 235: 1348 (1987). The activation of protein C by thrombin in the absence of thrombomodulin is poor.

Thrombin has also been shown to be a potent direct mitogen for a number of cell types, including cells of mesenchymal origin such as vascular smooth muscle cells. Chen, L.B. and Buchanan, J.M., Proc. Natl. Acad. Sci. USA, 72: 131 (1975). The direct interaction of thrombin

with vascular smooth muscle also results in vasoconstriction. Walz, D.A. et al., Proc. Soc. Expl. Biol. Med., 180: 518 (1985). Thrombin acts as a direct secretagogue inducing the release of a number of bioactive substances from vascular endothelial cells including tissue plasminogen activator. Levin, E.G. et al., Thromb. Haemost., 56: 115 (1986). In addition to these direct effects on vascular cells, the enzyme can indirectly elaborate potent mitogenic activity on vascular smooth muscle cells by the release of several potent growth factors (e.g. platelet-derived growth factor and epidermal growth factor) from platelet α -granules following thrombin-induced activation. Ross, R., N. Engl. J. Med., 314: 408 (1986).

Many significant disease states are related to abnormal hemostasis. With respect to the coronary arterial vasculature, abnormal thrombus formation due to the rupture of an established atherosclerotic plaque is the major cause of acute myocardial infarction and unstable angina. Moreover, treatment of an occlusive coronary thrombus by either thrombolytic therapy or percutaneous transluminal coronary angioplasty (PTCA) is often accompanied by an acute thrombotic reclosure of the affected vessel which requires immediate resolution. With respect to the venous vasculature, a high percentage of patients undergoing major surgery in the lower extremities or the abdominal area suffer from thrombus formation in the venous vasculature which can result in reduced blood flow to the affected extremity and a predisposition to pulmonary embolism. Disseminated intravascular coagulopathy commonly occurs within both vascular systems during septic shock, certain viral infections and cancer and is characterized by the rapid consumption of coagulation factors and systemic coagulation which results in the formation of life-threatening thrombi occurring throughout the vasculature leading to widespread organ failure.

Pathogenic thrombosis in the arterial vasculature is a major clinical concern in today's medicine. It is the leading cause of acute myocardial infarction which is one of the leading causes of death in the western world. Recurrent arterial thrombosis also remains one of the leading causes of failure following enzymatic or mechanical recanalization of occluded coronary vessels using thrombolytic agents or percutaneous transluminal coronary angioplasty (PTCA), respectively. Ross, A.M.,
10 Thrombosis in Cardiovascular Disorder, p. 327, W.B. Saunders Co. (Fuster, V. and Verstraete, M. edit. 1991); Califf, R.M. and Willerson, J.T., *Id.* at p 389. In contrast to thrombotic events in the venous vasculature, arterial thrombosis is the result of a complex interaction between fibrin formation resulting from the blood
15 coagulation cascade and cellular components, particularly platelets, which make up a large percentage of arterial thrombi. There is currently no effective therapy for the treatment or prevention of acute arterial thrombosis or rethrombosis since heparin, the most
20 widely used clinical anticoagulant administered i.v., has not been shown to be universally effective in this setting. Prins, M.H. and Hirsh, J., J. Am. Coll. Cardiol., 67: 3A (1991).

25 Besides the unpredictable, recurrent thrombotic reocclusion which commonly occurs following PTCA, a profound restenosis of the recanalized vessel occurs in 30 to 40% of patients 1 to 6 months following this procedure. Califf, R.M. et al., J. Am. Coll. Cardiol., 17: 2B
30 (1991). These patients require further treatment with either a repeat PTCA or coronary artery bypass surgery to relieve the newly formed stenosis. Restenosis of a mechanically damaged vessel is not a thrombotic process but instead is the result of a hyperproliferative
35 response in the surrounding smooth muscle cells which over time results in a decreased luminal diameter of the affected vessel due to increased muscle mass. *Id.* As

for arterial thrombosis, there is currently no effective pharmacologic treatment for the prevention of vascular restenosis following mechanical recanalization.

The need for safe and effective therapeutic anticoagulants has in one aspect focused on the role of factor Xa as the catalyst for the penultimate step in the blood coagulation cascade which is the formation of the serine protease thrombin.

Most preferred natural substrates for thrombin are reported to contain an uncharged amino acid in the P3 recognition subsite. For example, the thrombin cleavage site on the A α chain of fibrinogen, which is the primary physiological substrate for thrombin, is reported to contain a glycine residue in this position while the cleavage site on the B β chain contains a serine, as shown below:

P3	P2	P1	P1'	
-Gly-Val-Arg/Gly				Fibrinogen A α Chain
-Ser-Ala-Arg/Gly				Fibrinogen B β Chain

Peptidyl derivatives having an uncharged residue in the P3 position which is believed to bind to the active site of thrombin and thereby inhibit the conversion of fibrinogen to fibrin and cellular activation have been reported. Additionally, these derivatives have either an aldehyde, chloromethyl ketone or boronic acid functionality associated with the P1 amino acid. For example, substrate-like peptidyl derivatives such as D-phenylalanyl-prolyl-argininal (D-Phe-Pro-Arg-al), D-phenylalanyl-prolyl-arginine-chloromethyl ketone (P-PACK) and acetyl-D-phenylalanyl-prolyl-boroarginine (Ac-(D-Phe)-Pro-boroArg) have been reported to inhibit thrombin by directly binding to the active site of the enzyme. Bajusz, S., Symposia Biologica Hungarica, 25: 277 (1984), Bajusz, S. et al., J. Med. Chem., 33: 1729 (1990) and Bajusz, S. et al., Int. J. Peptide Protein Res. 12: 217 (1970); Kettner, C. and Shaw, E., Methods Enzymol., 80: 826 (1987); Kettner, C. et al., EP 293,881

(published December 7, 1988); Kettner, C., et al., J. Biol. Chem., 265: 18209 (1990). These molecules have been reported to be potent anticoagulants in the prevention of platelet-rich arterial thrombosis. Kelly, A.B. et al., Thromb. Haemostas., 65: 736 at abstract 257 (1991).

Peptidyl compounds which are said to be active site inhibitors of thrombin but which are said to differ in structure to those containing a uncharged amino acid in the P3 recognition subsite have been reported. The compound, Argatroban (also called 2R,4R-4-methyl-1-[N-2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-argininal]-2-piperidinecarboxylic acid), is also reported to bind directly to the active site of thrombin and has been thought to be the most potent and selective compound in the class of non-peptidyl inhibitors of this enzyme. Okamoto, S. et al., Biochem. Biophys. Res. Commun., 101: 440 (1981). Argatroban has been reported to be a potent antithrombotic agent in several experimental models of acute arterial thrombosis. Jang, I.K. et al., in both Circulation, 81: 219 (1990) and Circ. Res., 67: 1552 (1990).

Peptidyl compounds which are said to be inhibitors of thrombin and whose mode of action is thought to be by binding to the active site and another site on the enzyme have been reported. Hirudin and its various peptidyl derivatives have been reported to inhibit both conversion of fibrinogen to fibrin and platelet activation by binding to either the active site and exo site, or exo site only, of thrombin. Markwardt, F., Thromb. Haemostas., 66: 141 (1991). Hirudin is reported to be a 65 amino acid polypeptide originally isolated from leech salivary gland extracts. It is said to be one of the most potent inhibitors of thrombin known. Marki, W.E. and Wallis, R.B., Thromb. Haemostas., 64: 344 (1990). It is reported to inhibit thrombin by binding to both its anion-binding exo-site and to its catalytic active

site which are distinct and physically distant from each other. Rydel, supra. Hirudin has been reported to be a potent antithrombotic agent in vitro. Markwardt, F. et al., Pharmazie, 43: 202 (1988); Kelly, A.B. et al.,
5 Blood, 77: 1 (1991). In addition to its antithrombotic effects, hirudin has been reported to also effectively inhibit smooth muscle proliferation and the associated restenosis following mechanical damage to a atherosclerotic rabbit femoral artery. Sarembock, I.J. et al.,
10 Circulation, 84: 232 (1991).

Hirugen has been reported to be a peptide derived from the anionic carboxy-terminus of hirudin. It is reported to bind only to the anion binding exo-site of thrombin and thereby inhibit the formation of fibrin but
15 not the catalytic turnover of small synthetic substrates which have access to the unblocked active site of the enzyme. Maraganore, J.M. et al., J. Biol. Chem., 264: 8692 (1989); Naski, M.C. et al., J. Biol. Chem., 265: 13484 (1990). The region of hirudin represented by
20 hirugen has been reported, as according to by x-ray crystallographic analysis, to bind directly to the exo site of thrombin. Skrzypczak-Jankun, E. et al., Thromb. Haemostas., 65: 830 at abstract 507 (1991). Moreover, the binding of hirugen has also been reported to enhance
25 the catalytic turnover of certain small synthetic substrates by thrombin, indicating that a conformational change in the enzyme active site may accompany occupancy of the exo-site. Liu, supra. Hirugen also is reported to block thrombin-mediated platelet aggregation.
30 Jakubowski, J.A. and Maraganore, J.M., Blood, 75: 399 (1990).

Hirulog has been reported to be a synthetic chimeric molecule comprised of a hirugen-like sequence linked by a spacer region to the peptide, D-phenyl-
35 alanyl-prolyl-arginine which is based on a preferred substrate recognition site for thrombin. The hirugen-like sequence is said to be linked to this peptide

through the C-terminal end of the peptide. Maraganone, J.M. et al., *Biochemistry*, 29: 7095 (1990). Hirulog has been reported to be an effective antithrombotic agent in preventing both fibrin-rich and platelet-rich thrombo-
5 sis. Maraganone, J.M. et al., *Thromb. Haemostas.*, 65: 651 at abstract 17 (1991).

Cyclotheonamide A and B, isolated from the marine sponge, *Theonella*, a genus of marine sponges, have been reported to be inhibitors of thrombin with an IC₅₀ of
10 0.076 mg/mL. Structurally, they have been characterized as cyclic peptides containing an α -keto amide moiety. Fusetani et al., *J. Am. Chem. Soc.* 112: 7053-7054 (1991) and Hagihara et al., *J. Am. Chem. Soc.*, 114: 6570-6571 (1992). It has been proposed that the α -keto group of
15 the cyclotheonamides may function as an electrophilic mimic of the Arg-X scissile amide bond of the thrombin substrates. Hagihara et al., *Id.* at 6570. The partial synthesis of cyclotheonamide A and the total synthesis of cyclotheonamide B have been reported. Wipf et al.,
20 *Tetrahedron Lett.*, 33: 4275-4278 (1992) and Hagihara et al., *J. Am. Chem. Soc.*, 114: 6570-6571 (1992).

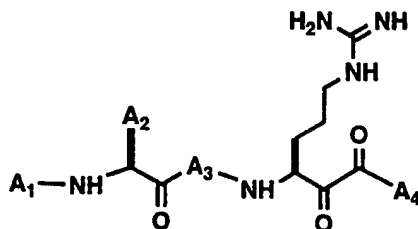
α -Keto ester derivatives of N-protected amino acids and peptides have been reported as inhibitors of serine proteases, as neutrophil elastase and cathepsin. G.
25 Mehdi et al., *Biochem. Biophys. Res. Commun.*, 166: 595-600 (1990) and Angelastro et al., *J. Med. Chem.*, 33: 11-13 (1990).

Alpha keto-amide derivatives of amino acids and peptides have been reported to be inhibit proteases.
30 For example, fluoro-substituted keto amide derivatives have been reported to be inhibitors of proteases. European Patent Application No. 275,101 (published July 20, 1988). L-valyl-L-valyl-3-amino-2-oxovaleryl-D-leucyl-L-valine had been reported to be an inhibitor of
35 prolyl endopeptidase. Nagai et al., *J. Antibiotics*, 44: 956-961 (1991). 3-Amino-2-oxo-4-phenylbutanoic acid amide has been reported to be an inhibitor of arginyl

aminopeptidase (with inhibitor constant of 1.5 mM),
 cytosol aminopeptidase (with inhibitor constant of 1.0
 mM) and microsomal aminopeptidase (with inhibitor con-
 stant of 2.5 mM). Ocain et al., J. Med. Chem., 35: 451-
 5 456 (1992). 2-Oxo-2-(pyrrolidin-2yl) acetyl derivatives
 have been reported to be inhibitors of prolyl endopepti-
 dase. Someno et al., European Patent Application No.
 468,339 (published January 29, 1992). Certain alpha
 keto-amide derivatives of peptides have been reported to
 10 inhibit various serine and cysteine proteases. Powers
 J.C., International Application No. WO 92/12140
 (published July 23, 1992).

Summary of the Invention

15 The present invention in one aspect is directed to
 novel compounds which are useful as in vitro inhibitors
 of coagulation proteases and as in vivo antithrombic
 agents. These compounds have the structure:



(I)

wherein

(a) A₁ is selected from the group consisting of
 R₁-C(O)-, R₁-O-C(O)-, R₁-NH-C(O)-, R₁-S(O₂)-, R₁-O-
 25 S(O₂)-, and R₁-NH-S(O₂)-, wherein R₁ is selected from
 the group consisting of

alkyl of 1 to about 12 carbon atoms,

alkenyl of about 3 to about 6 carbon atoms,

aryl of about 6 to about 14 carbon atoms which

30 is optionally mono-substituted with X₁ or optionally di-
 substituted with X₁ and X₂,

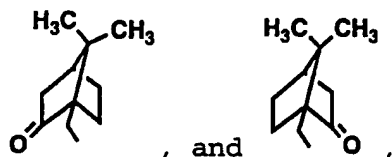
13

aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X_1 or optionally di-substituted with X_1 and X_2 ,

5 aralkenyl of about 8 to about 15 carbon atoms which is optionally mono-substituted with X_1 or optionally di-substituted with X_1 and X_2 ,

perfluoroalkyl of 1 to about 12 carbon atoms,
perfluoroaryl of about 6 to about 14 carbon atoms,

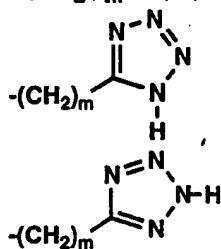
10 trimethylsilylalkyl of 4 to about 8 carbon atoms,



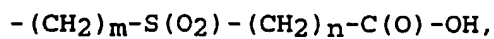
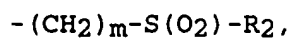
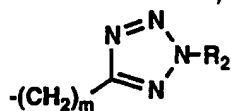
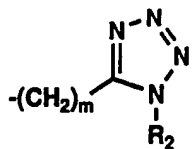
wherein X_1 and X_2 are independently selected from the group consisting of bromo, chloro, fluoro, Y_1 -, HO -, Y_1 -
15 O -, NH_2 -, Y_1-NH -, $(Y_1, Y_2)N$ -, $Y_1-C(O)-NH$ -, HS -, Y_1-S -,
 $Y_1-S(O)$ -, $Y_1-S(O_2)$ -, $HO-S(O_2)$ -, $Y_1-O-S(O_2)$ -, $NH_2-S(O_2)$ -
and $Y_1-NH-S(O_2)$ -, wherein Y_1 and Y_2 are independently
selected from the group consisting of trifluomethyl,
pentafluoroethyl, alkyl of 1 to about 12 carbon atoms,
20 aryl of about 6 to about 14 carbon atoms, and aralkyl of
about 6 to about 15 carbon atoms;

(b) A_2 is selected from the group consisting of
hydrogen,

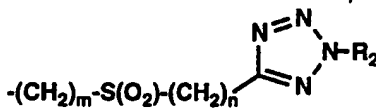
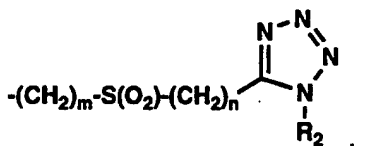
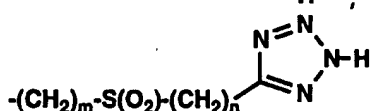
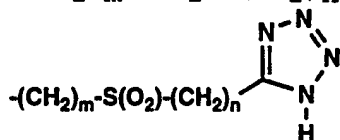
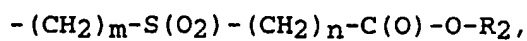
25 R_2 -,
- $(CH_2)_m-C(O)-O-H$,
- $(CH_2)_m-C(O)-O-R_2$,



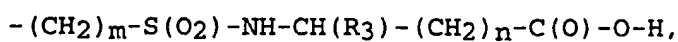
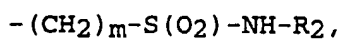
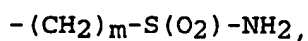
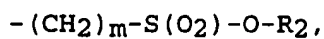
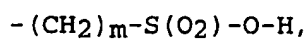
14



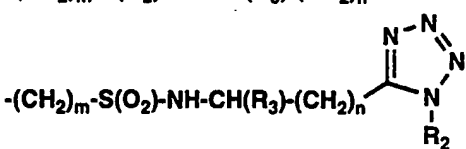
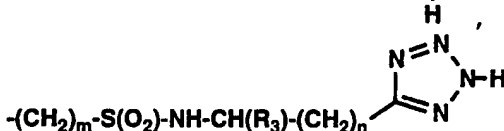
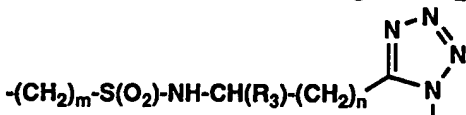
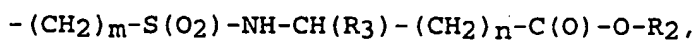
5



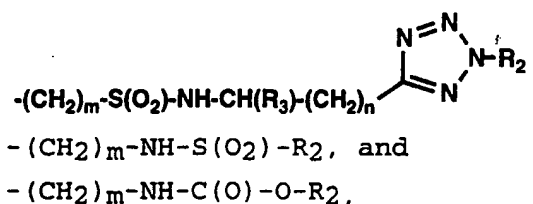
10



15

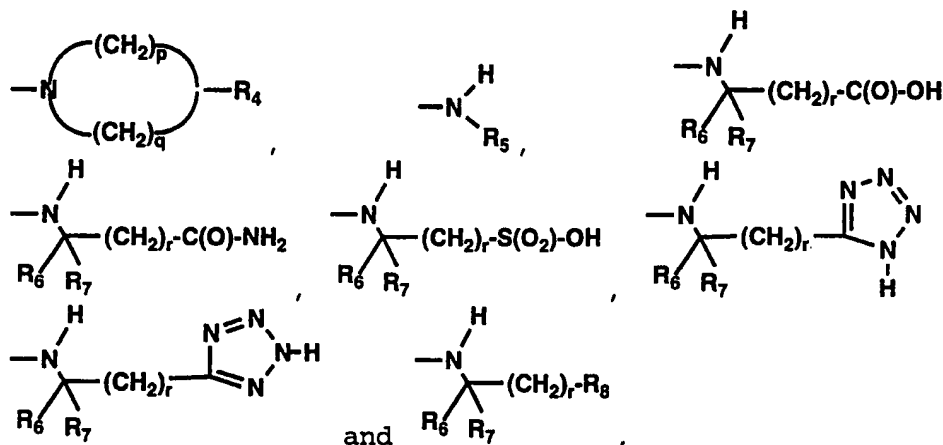


SUBSTITUTE SHEET



wherein

- 5 (i) m is 1, 2 or 3;
 (ii) n is 0, 1, 2, 3 or 4;
 (iii) R₂ is selected from the group consisting of alkyl of 1 to about 12 carbon atoms, alkenyl of about 3 to about 6 carbon atoms, aryl of about 6 to
 10 about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms and aralkenyl of about 8 to about 15 carbons atoms; and
 (iv) R₃ is selected from the group consisting of hydrogen, alkyl of 1 to about 4 carbon atoms,
 15 aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms, and alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -OH, -C(O)-OH, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃ and -NH-S(O₂)-
 20 CH₃;
- (c) A₃ is an amino acid residue of an amino acid selected from the group consisting of L-alanine, L-azetidinecarboxylic acid, glycine, L-isoleucine, L-leucine, L-lysine mono-substituted at its ε-amino group
 25 with R₂-S(O₂)-, L-methionine sulfone, N-methylglycine, L-ornithine mono-substituted at its δ-amino group with R₂-S(O₂)-, L-pipecolic acid, L-phenylalanine, L-proline, L-valine, and trans-4-hydroxy-L-proline wherein R₂ is as defined hereinabove; and
- 30 (d) A₄ is selected from the group consisting of



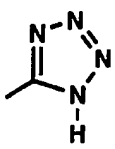
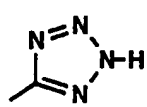
wherein

- 5 (i) p and q are each independently selected integers from 1 to 5, wherein the sum of p + q is 4 to 8;
- (ii) R₄ is aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, alkoxy of 1 to about 4 carbon atoms, -NH₂, -C(O)-OH, -C(O)-NH₂, fluoro, -OH, -NO₂ and -CF₃;
- 10 (iii) R₅ is aryl of about 6 to 14 carbon atoms;
- 15 (iv) R₆ is selected from the group consisting of hydrogen and alkyl of 1 to about 4 carbon atoms;
- (v) R₇ is selected from the group consisting of
- 20 hydrogen;
- alkyl of 1 to about 4 carbon atoms;
- aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH₂, -C(O)-OH, -C(O)-NH₂, fluoro, -OH, -NO₂, -CF₃, alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms;
- 25

aralkyl of about 6 to about 15 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH₂, -C(O)-OH, -C(O)-NH₂, fluoro, -OH, -NO₂, -CF₃, alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms; and

alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -OH, -C(O)-OH, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and -NH-S(O₂)-CH₃; and

(vi) R₈ is selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, and aralkyl of about 6 to about 15 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, wherein X₃ and X₄ are independently selected from the group consisting of -C(O)-OH,

-S(O₂)-OH,  and ; and

(vii) r is 0, 1, 2 or 3; and pharmaceutically acceptable salts thereof.

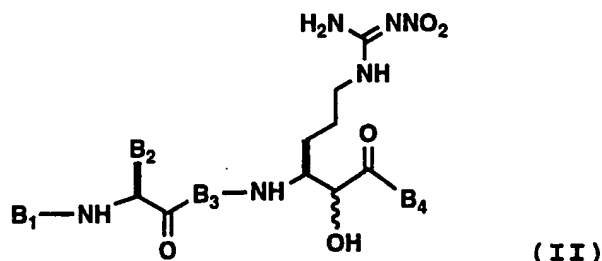
In another aspect, the present invention is directed to a pharmaceutical composition for treating coagulation disorders which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound of the present invention.

In yet another aspect, the present invention is directed to methods of preventing or treating in a mammal a condition characterized by abnormal thrombus formation.

One aspect of the present invention allows the stereoselective synthesis which yields the optically pure arginine ketoamides of formula I. Thus, in another

aspect, the present invention is directed to intermediates useful for the preparation of the compounds of the present invention. These intermediates have the structure:

5



wherein

(a) B₁ is selected from the group consisting of R₉-C(O)-, R₉-O-C(O)-, R₉-NH-C(O)-, R₉-S(O₂)-, R₉-O-S(O₂)- and R₉-NH-S(O₂)-, wherein R₉ is selected from the group consisting of

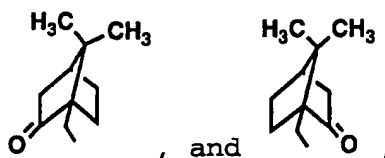
alkyl of 1 to about 12 carbon atoms,
alkenyl of about 3 to about 6 carbon atoms,
aryl of about 6 to about 14 carbon atoms which is optionally mono-substituted with X₅ or optionally di-substituted with X₅ and X₆,

aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X₅ or optionally di-substituted with X₅ and X₆,

aralkenyl of about 8 to about 15 carbon atoms which is optionally mono-substituted with X₅ or optionally di-substituted with X₅ and X₆,

perfluoroalkyl of 1 to about 12 carbon atoms,
perfluoroaryl of about 6 to about 14 carbon atoms,

trimethylsilylalkyl of 4 to about 8 carbon atoms,



wherein X₅ and X₆ are independently selected from the group consisting of bromo, chloro, fluoro, Y₃-, Y₃-O-, Y₃-O-C(O)-NH-, Y₃-O-C(O)-N(Y₄)-, (Y₃,Y₄)N-, Y₃-C(O)-NH-, Y₃-S-, Y₃-S(O)-, Y₃-S(O₂)-, Y₃-O-S(O₂)-, NH₂-S(O₂)- and
 5 Y₃-NH-S(O₂)-, wherein Y₃ and Y₄ are independently selected from the group consisting of trifluoromethyl, pentafluoroethyl, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms and alkyl of 1 carbon atom to about 12 which is optionally
 10 mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms;

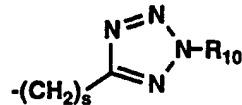
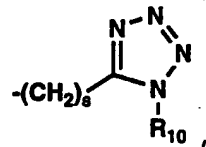
(b) B₂ is selected from the group consisting of

hydrogen,

15

R₁₀-,

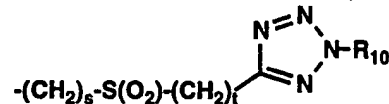
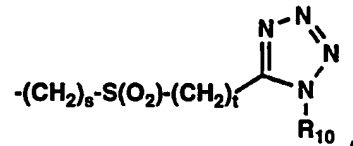
-(CH₂)_s-C(O)-O-R₁₀,



-(CH₂)_s-S(O₂)-R₁₀,

20

-(CH₂)_s-S(O₂)-(CH₂)_t-C(O)-O-R₁₀,



-(CH₂)_s-S(O₂)-O-R₁₀,

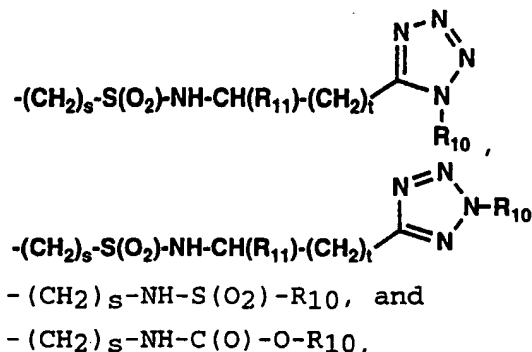
-(CH₂)_s-S(O₂)-NH-C(O)-O-R₁₀,

25

-(CH₂)_s-S(O₂)-NH-R₁₀,

-(CH₂)_s-S(O₂)-NH-CH(R₁₁)-(CH₂)_t-C(O)-O-R₁₀,

20



5 wherein

(i) s is 1, 2 or 3;

(ii) t is 0, 1, 2, 3 or 4;

(iii) R₁₀ is selected from the group

consisting of alkenyl of about 3 to about 6 carbon
 10 atoms, aryl of about 6 to about 14 carbon atoms, aralkyl
 of about 6 to about 15 carbon atoms, aralkenyl of about
 8 to about 15 carbons atoms and alkyl of 1 to about 12
 carbon atoms which is optionally mono-substituted with
 aralkyloxy of about 6 to about 15 carbon atoms; and

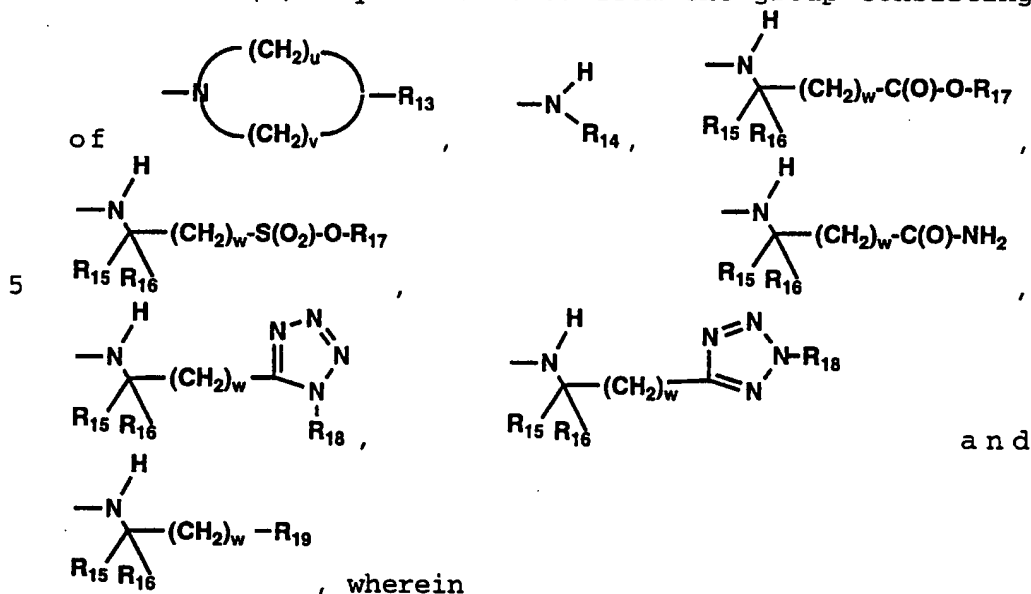
15 (iv) R₁₁ is selected from the group
 consisting of hydrogen, alkyl of 1 to about 4 carbon
 atoms, aryl of about 6 to about 14 carbon atoms, aralkyl
 of about 6 to about 15 carbon atoms, and alkyl of 1 to
 about 4 carbon atoms substituted with a substituent
 20 selected from the group consisting of -O-R₁₀, -C(O)-O-
 R₁₀, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃ and -NH-
 S(O₂)-CH₃;

(c) B₃ is an amino acid residue of an amino
 acid selected from the group consisting of L-alanine, L-
 25 azetidinecarboxylic acid, glycine, L-isoleucine, L-
 leucine, L-lysine mono-substituted at its ε-amino group
 with R₂-S(O₂)-, L-methionine sulfone, N-methylglycine,
 L-ornithine mono-substituted at its δ-amino group with
 R₂-S(O₂)-, L-pipecolic acid, L-phenylalanine, L-proline,
 30 L-valine, and trans-4-hydroxy-L-proline substituted at
 4-hydroxy group with R₁₂-O-C(O)-, wherein R₁₂ is
 selected from the group consisting of alkyl of 1 to

21

about 12 carbon atoms and aralkyl of about 6 to about 15 carbon atoms; and

(d) B₄ is selected from the group consisting



(i) u and v are each independently
10 selected integers from 1 to 5, wherein the sum of u + v
is 4 to 8;

(ii) R₁₃ is aryl of about 6 to about
14 carbon atoms which is optionally substituted with 1
or 2 substituents each independently selected from the
15 group consisting of alkyl of 1 to about 4 carbon atoms,
alkoxy of 1 to about 4 carbon atoms, -NH-C(O)-O-X₇,
-C(O)-O-X₇, -C(O)-NH₂, fluoro, -O-X₇, -NO₂ and -CF₃;

(iii) R₁₄ is aryl of about 6 to about
14 carbon atoms;

20 (iv) R₁₅ is selected from the group
consisting of hydrogen and alkyl of 1 to about 4 carbon
atoms;

(v) R₁₆ is selected from the group
consisting of

25 hydrogen,
alkyl of 1 to about 4 carbon
atoms,

aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH-C(O)-O-X₈, -C(O)-O-X₈, -C(O)-NH₂,
5 fluoro, -O-X₈, -NO₂, -CF₃, alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms,

aralkyl of about 6 to about 15 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH-C(O)-O-X₉, -C(O)-O-X₉, -C(O)-NH₂,
10 fluoro, -O-X₉, -NO₂, -CF₃, alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms, and

alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -O-X₁₀, -C(O)-O-X₁₀, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and -NH-S(O₂)-CH₃;

(vii) R₁₇ is selected from the group consisting of alkyl of 1 to about 4 carbon atoms and
20 aralkyl of about 6 to 15 carbon atoms;

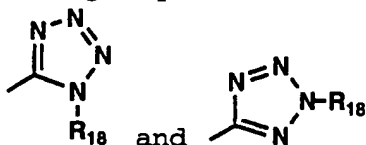
(viii) R₁₈ is alkyl of 1 to about 12 carbon atoms which is optionally mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms;

(ix) R₁₉ is selected from the group consisting of hydrogen, aryl of about 6 to about 14 carbon atoms which is optionally mono-substituted with X₁₁ or optionally di-substituted with X₁₁ and X₁₂; and
25 aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X₁₁ or optionally di-substituted with X₁₁ and X₁₂; and
30

(x) w is 0, 1, 2, 3, 4 or 5;

wherein X₇, X₈, X₉ and X₁₀ are independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms and aralkyl of about 6 to about 15 carbon atoms;
35 and wherein X₁₁ and X₁₂ are independently selected from

the group consisting of $-C(O)-O-R_{17}$, $-S(O_2)-O-R_{17}$,



Definitions

5 In accordance with the present invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise.

The term "amino acid" refers to both natural and unnatural amino acids in either their L- or D- forms.

10 Natural amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. For example, unnatural

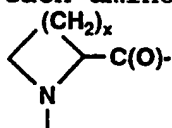
15 amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-amino-adipic acid, β -alanine, aminopropionic acid, 2-amino-butyrac acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-

20 aminoisobutyric acid, 2-aminopimelic acid, 2,4 diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-

25 isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, ornithine and pipecolic acid.

The term "amino acid residue" refers to $-NH-CH(R)-CO-$, wherein R is the side chain group distinguishing

30 each amino acid. For cyclic amino acids, the residue is



, wherein x is 1, 2 or 3 representing the

azetidinecarboxylic acid, proline or pipecolic acid residues, respectively.

The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched-chain and
5 cyclic groups.

The term "alkenyl" refers to unsaturated hydrocar-
byl groups which contain at least one carbon-carbon
double bond and includes straight-chain, branched-chain
and cyclic groups.

10 The term "aryl" refers to aromatic groups which
have at least one ring having a conjugated pi electron
system and includes carbocyclic aryl, heterocyclic aryl
and biaryl groups, all of which may be optionally
substituted.

15 The term "aralkyl" refers to an alkyl group substi-
tuted with an aryl group. Suitable aralkyl groups
include benzyl, picolyl, and the like, all of which may
be optionally substituted.

The term "aralkenyl" refers to an alkenyl group sub-
stituted with an aryl group. Suitable aralkenyl groups
20 include styrenyl and the like, all of which may be
optionally substituted.

The term "alkoxy" refers to the group -OR wherein R
is alkyl.

25 The term "alkenyloxy" refers to the group -O-R
wherein R is alkenyl.

The term "aryloxy" refers to the group -O-R wherein
R is aryl.

The term "aralkyloxy" refers to the group -O-R
30 wherein R is aralkyl.

The term "alkylene" refers to a divalent straight
chain or branched chain saturated aliphatic radical.

The term "alkylenecarboxy" refers to the group
-alk-COOH where alk is alkylene.

35 The term "carboxamide" refers to the group -C(O)-
NH₂.

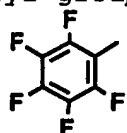
The term "alkylenecarboxamide" refers to the group -alk-C(O)NH₂ where alk is alkylene.

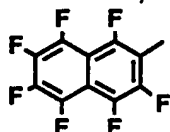
The term "alkylenehydroxy" refers to the group -alk-OH wherein alk is alkylene.

5 The term "methylene" refers to -CH₂-.

The term "perfluoroalkyl refers to an alkyl group wherein each hydrogen is replaced by a fluoro. Suitable perfluoroalkyl groups include perfluoromethyl (having the structure of CF₃-) and perfluroethyl (having the structure of CF₃-CF₂-) and the like.

The term "perfluoroaryl refers to an aryl group wherein each hydrogen is replaced by a fluoro. Suitable perfluoroaryl groups include perfluorophenyl (having the

formula of ) and 2-perfluoronaphthyl (having the

15 formula of ), and the like.

In addition, the following abbreviations stand for the following:

"Bn" refers to benzyl.

"Boc" refers to t-butoxycarbonyl.

20 "Boc₂O" refers di-t-butyldicarbonate.

"BocAsp^{Bn}-OH" refers to N-Boc-L-aspartic acid-(β-benzyl ester).

"BocPro-OH" refers to N-Boc-L-proline.

"Bom" refers to benzyloxymethyl.

25 "BOP" refers to benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate.

"Brine" refers to an aqueous saturated solution of sodium chloride.

"CH₂Cl₂" refers to dichloromethane.

30 "CH₃CN" refers to acetonitrile.

"DCA" refers to dichloroacetic acid.

"DMF" refers to dimethylformamide.

"DMSO" refers to dimethylsulfoxide.

"EDC" refers to ethyl-3-(3-dimethylamino)-propyl-carbodiimide hydrochloride salt.

"HOBt" refers to 1-hydroxybenzotriazole.

5 "HCl" refers to hydrochloric acid.

"HF" refers to hydrofluoric acid.

"HPLC" refers to high pressure liquid chromatography.

"KOH" refers to potassium hydroxide.

10 "MeOH" refers to methanol.

"NaCO₃" refers to sodium carbonate.

"NEt₃" refers to triethylamine.

"NMM" refers to 4-methylmorpholine.

"Pd/C" refers to palladium on carbon.

15 "PhMe" refers to toluene.

"POCl₃" refers to phosphorous oxychloride

"TFA" refers to trifluoroacetic acid.

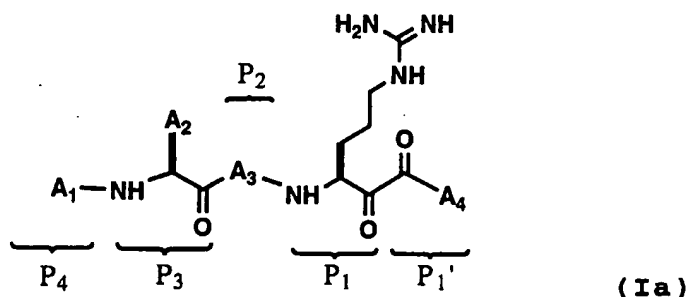
Brief Description of the Drawings

20 Figure 1, i represents potassium cyanide, potassium bicarbonate, water; ii represents HCl/water/dioxane; iii represents dry HCl/methanol; iv represents Boc₂O/THF/NaHCO₃/H₂O; v represents lithium hydroxide/methanol/water; vi represents Dowex-50 acid form;
25 vii represents B₄-NH₂/BOP/DMF where B₄ is as defined in connection with formula I; viii represents TFA/methylene chloride; ix represents Boc-Pro-OH/BOP/DMF; x represents Boc-Asp^{Bn}-OH/BOP/DMF; xi represents modified Moffatt conditions; and xii represents either H₂/Pd on carbon or
30 HF/anisole.

Detailed Description of the Invention

Preferred Compounds

35 The compounds of the present invention can be divided conceptually into parts as shown in the following formula Ia:



P₁ corresponds to an arginine residue. P₂ corresponds to an amino acid residue such as a proline residue, trans-4-hydroxyproline residue, glycine residue, isoleucine residue and other of the above-specified amino acid residues. P₃ corresponds to an amino acid residue such as an aspartic acid residue, aspartic acid ester residue, glycine amino acid residue, glutamic acid residue, glutamic acid ester residue, methionine sulfone residue, alanine residue which is β -substituted with a substituted or unsubstituted tetrazole, and other of the above-specified amino acids or derivatives thereof. In a particular compound, the A₁ and A₄ groups for P₄ and P₁' respectively are selected depending on the specific enzyme to be selectively inhibited.

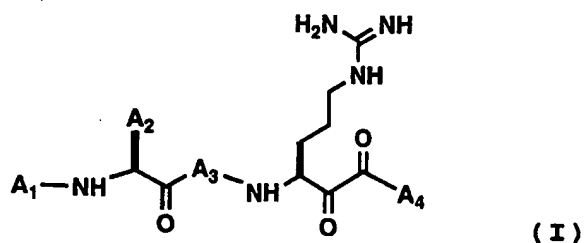
Among other factors, in one aspect, the present invention is based on our surprising finding that the compounds of formula I which include at the P₃ position an aspartic acid residue, suitable ester derivative of aspartic acid, methionine sulfone residue or alanine residue β -substituted with a substituted or unsubstituted tetrazole are highly active inhibitors of *in vitro* coagulation. Certain of these compounds exhibit IC₅₀'s in an assay of thrombin inhibition of less than 10 nM. (See Example A).

The compounds of formula I possessing a negative charge at physiological pH are thought to have specific advantages as *in vivo* antithrombotic agents. The negative charge may be incorporated into these molecules in several ways. Compounds of formula I which include at

the P₃ position the aspartic acid residue or an alanine residue β -substituted with an unsubstituted tetrazole directly possess such a negative charge. Compounds of formula I having at the P₃ position, a methionine sulfone residue, suitable ester derivatives of aspartic acid or suitable derivatives of the alanine residue β -substituted with a substituted tetrazole derivatives, may be advantageously derivatized elsewhere with an ionizable group which at physiological pH would yield a negative charge. Such negatively charged groups would include the carboxy group, sulfonate group or unsubstituted tetrazole which in one approach may be conveniently introduced at the P₁' position.

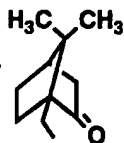
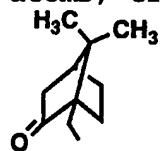
Suitable ester derivatives of aspartic acid include those that can be cleaved in vivo to yield the corresponding aspartic acid derivative. Such esters are believed to exhibit improved bioavailability and to have a longer half life in the circulation. The preferred compounds of formula I have A₁ groups which preferably comprise hydrophobic groups which have been selected so as to enhance potency and/or selectivity of these compounds.

Compounds of the present invention include those represented by formula I.



The compounds of the present invention include those wherein A₁ is R₁-C(O)-, R₁-O-C(O)-, R₁-NH-C(O)-, R₁-S(O₂)-, R₁-O-S(O₂)- or R₁-NH-S(O₂)-. Preferred compounds include those wherein A₁ is R₁-C(O)-, R₁-O-C(O)- or R₁-S(O₂)-.

The compounds of the present invention include those wherein R₁ is an alkyl of 1 to about 12 carbon atoms; alkenyl of about 3 to about 6 carbon atoms; aryl of about 6 to about 14 carbon atoms which is optionally
 5 mono-substituted with X₁ or optionally di-substituted with X₁ and X₂; aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X₁ or optionally di-substituted with X₁ and X₂; aralkenyl of
 10 mono-substituted with X₁ or optionally di-substituted with X₁ and X₂; perfluoroalkyl of 1 to about 12 carbon atoms; perfluoroaryl of about 6 to about 14 carbon atoms; trimethylsilylalkyl of 4 to about 8 carbon atoms;



; or , wherein X₁ and X₂ are each inde-
 15 pendently selected from the group consisting of bromo, chloro, fluoro, Y₁-, HO-, Y₁-O-, NH₂-, Y₁-NH-, (Y₁,Y₂)N-, Y₁-C(O)-NH-, HS-, Y₁-S-, Y₁-S(O)-, Y₁-S(O₂)-, HO-S(O₂)-, Y₁-O-S(O₂)-, NH₂-S(O₂)- and Y₁-NH-S(O₂)-, wherein Y₁ and Y₂ are independently selected from the
 20 group consisting of trifluomethyl, pentafluoroethyl, alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms, and aralkyl of about 6 to about 15 carbon atoms.

Preferred compounds include those wherein R₁ is
 25 alkyl of 1 to about 12 carbon atoms; aryl of about 6 to about 14 carbon atoms which is optionally mono-substituted with X₁ or optionally di-substituted with X₁ and X₂; or aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X₁ or
 30 optionally di-substituted with X₁ and X₂. Suitable alkyl groups include methyl, ethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, 2,2-dimethylpropyl, butyl, 3-methylbutyl, 1-propylbutyl, pentyl, hexyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl,

adamantyl and adamantylmethyl. Suitable aryl groups include phenyl, naphthyl, biphenyl, 2-thienyl, 2-pyrrolyl and 2-furyl. Suitable aralkyl groups include phenylmethyl, diphenylmethyl, biphenyl, biphenylmethyl, naphthyl, naphthylmethyl, α -phenylmethylphenyl and 2-phenylethylene.

Especially preferred compounds include those wherein R_1 is 1,1-dimethylethyl, 2,2-dimethylpropyl, butyl, 3-methylbutyl, 1-propylbutyl, phenylmethyl or naphthyl.

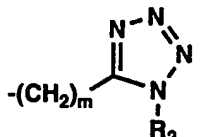
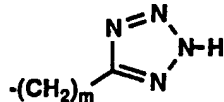
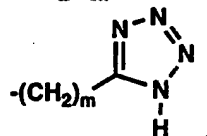
The compounds of the present invention include those wherein A_2 is

hydrogen ,

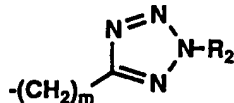
R_2 - ,

15 $-(CH_2)_m-C(O)-O-H$,

$-(CH_2)_m-C(O)-O-R_2$,



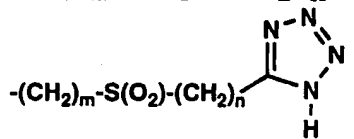
20



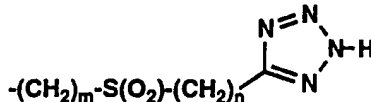
$-(CH_2)_m-S(O_2)-R_2$,

$-(CH_2)_m-S(O_2)-(CH_2)_n-C(O)-OH$,

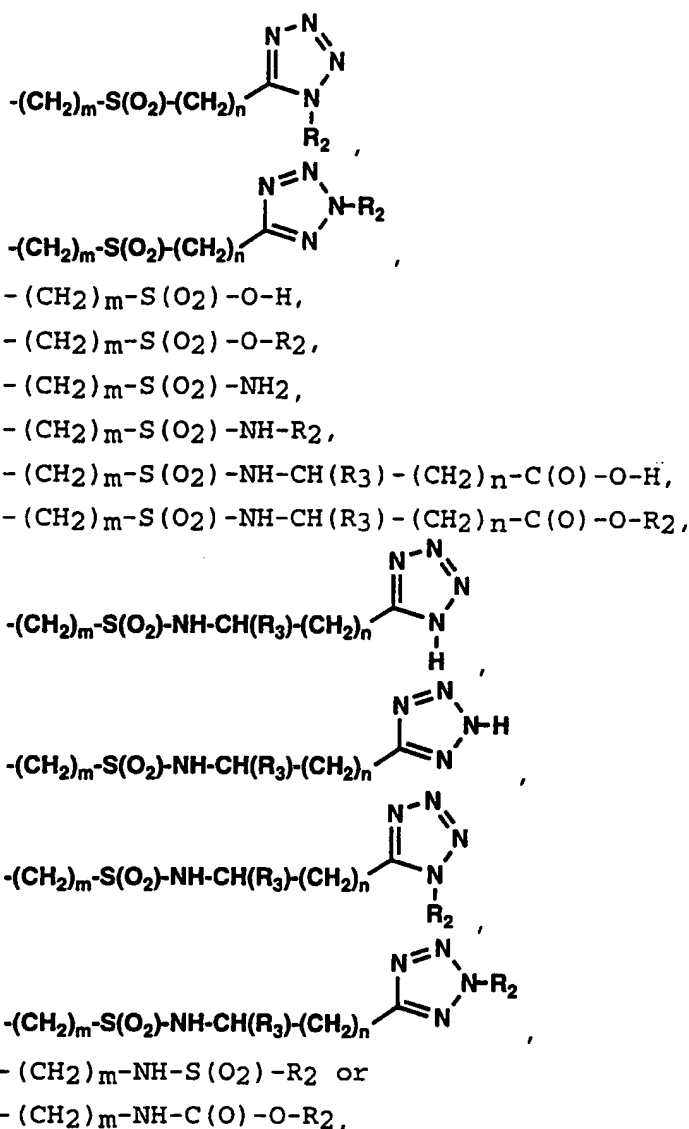
$-(CH_2)_m-S(O_2)-(CH_2)_n-C(O)-O-R_2$,



25



31

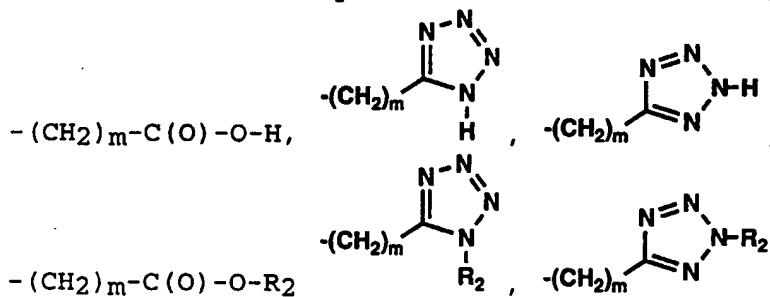


15 wherein m is 1, 2 or 3; n is 0, 1, 2, 3 or 4; R₂ is
 alkyl of 1 to about 12 carbon atoms, alkenyl of about 3
 to about 6 carbon atoms, aryl of about 6 to about 14
 carbon atoms, aralkyl of about 6 to about 15 carbon
 atoms, or aralkenyl of about 8 to about 15 carbons
 20 atoms; and R₃ is hydrogen, alkyl of 1 to about 4 carbon
 atoms, aryl of about 6 to about 14 carbon atoms, aralkyl
 of about 6 to about 15 carbon atoms, or alkyl of 1 to
 about 4 carbon atoms substituted with a substituent
 selected from the group consisting of -OH, -C(O)-OH,

$-C(O)-NH_2$, $-S-CH_3$, $-S(O)-CH_3$, $-S(O_2)-CH_3$ and $-NH-S(O_2)-CH_3$.

Suitable alkyl groups include methyl, ethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, 2,2-dimethylpropyl, butyl, 3-methylbutyl, 1-propylbutyl, pentyl, hexyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, adamantyl and adamantylmethyl. Suitable alkenyl groups include 2-propenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 5-hexenyl and 2-cyclopentenyl. Suitable aryl groups include phenyl, naphthyl, biphenyl, pyridyl, 2-thienyl, 2-pyrrolyl and 2-furyl. Suitable aralkyl groups include phenylmethyl, diphenylmethyl, biphenyl, biphenylmethyl, naphthyl, naphthylmethyl, α -phenylmethylphenyl and 2-phenylethylene.

Preferred compounds include those wherein A_2 is



Especially preferred compounds include those wherein m is 1 and if there is an R_2 , it is methyl.

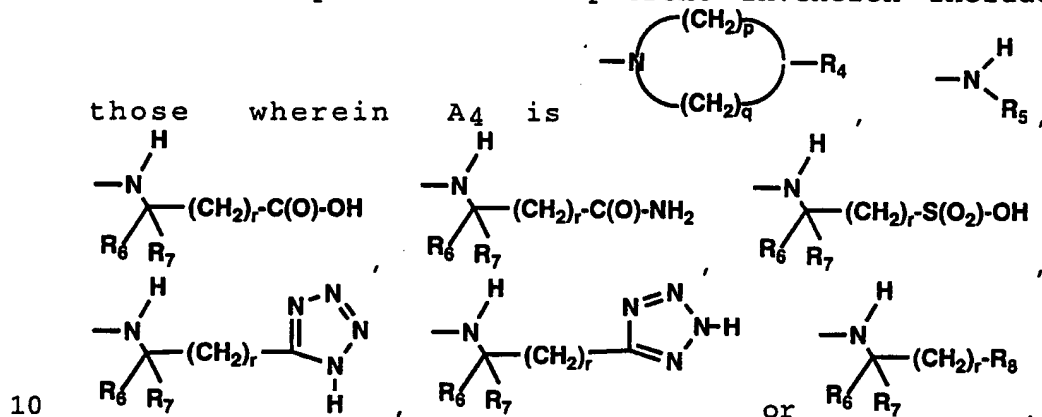
The compounds of the present invention include those wherein A_3 is an amino acid residue of L-alanine, L-azetidinecarboxylic acid, glycine, L-isoleucine, L-leucine, L-lysine mono-substituted at its ϵ -amino group with $R_2-S(O_2)-$, L-methionine sulfone, N-methylglycine, L-ornithine mono-substituted at its δ -amino group with $R_2-S(O_2)-$, L-pipecolic acid, L-phenylalanine, L-proline, L-valine, and trans-4-hydroxy-L-proline, wherein R_2 is alkyl of 1 to about 12 carbon atoms, alkenyl of about 3 to about 6 carbon atoms, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon

atoms and aralkenyl of about 8 to about 15 carbons atoms.

Preferred compounds include those wherein A₃ is glycine, L-isoleucine or proline.

5 Especially preferred compounds include those wherein A₃ is proline.

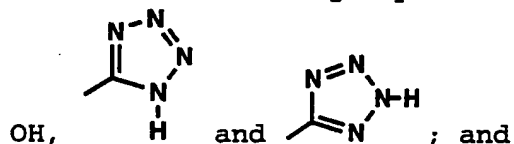
The compounds of the present invention include



- (i) p and q are each independently selected integers from 1 to 5, wherein the sum of p + q is 4 to 8;
- 15 (ii) R₄ is aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, alkoxy of 1 to about 4 carbon atoms, -NH₂, -C(O)-OH, -C(O)-NH₂,
 20 fluoro, -OH, -NO₂ and -CF₃;
- (iii) R₅ is aryl of about 6 to 14 carbon atoms;
- (iv) R₆ is selected from the group consisting of hydrogen and alkyl of 1 to about 4 carbon atoms;
- (v) R₇ is selected from the group consisting
 25 of hydrogen; alkyl of 1 to about 4 carbon atoms; aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH₂, -C(O)-OH, -C(O)-NH₂, fluoro, -OH, -NO₂, -CF₃, alkyl of 1 to about
 30 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms;

aralkyl of about 6 to about 15 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH₂, -C(O)-OH, -C(O)-NH₂, fluoro, -OH, -NO₂, -CF₃,
 5 alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms; and alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -OH, -C(O)-OH, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and -NH-S(O₂)-CH₃; and

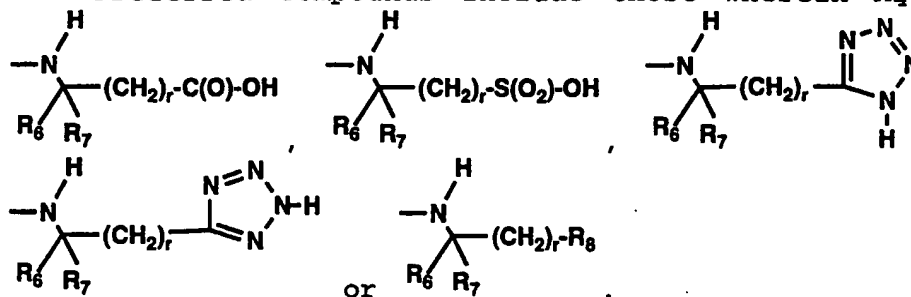
10 (vi) R_g is selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, and
 15 aralkyl of about 6 to about 15 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, wherein X₃ and X₄ are independently selected from the group consisting of -C(O)-OH, -S(O₂)-



(vii) r is 0, 1, 2 or 3

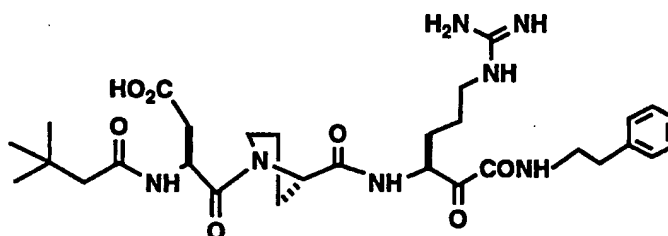
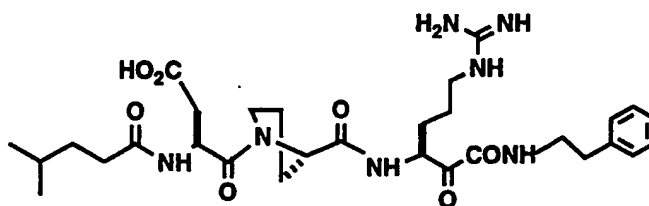
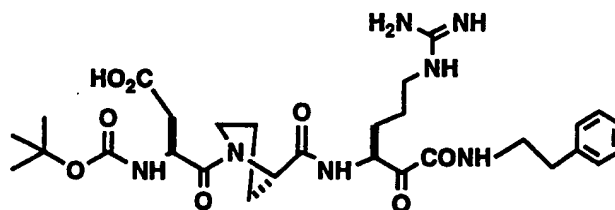
20 Suitable alkyl groups include methyl, ethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, 2,2-dimethylpropyl, butyl, 3-methylbutyl, 1-propylbutyl, pentyl, hexyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, adamantyl and adamantylmethyl.
 25 Suitable alkenyl groups include 2-propenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 5-hexenyl and 2-cyclopentenyl. Suitable aryl groups include phenyl, naphthyl, biphenyl, pyridyl, 2-thienyl, 2-pyrrolyl and 2-furyl. Suitable aralkyl groups include phenylmethyl, diphenylmethyl,
 30 biphenyl, biphenylmethyl, naphthyl, naphthylmethyl, α-phenylmethylphenyl and 2-phenylethylene. Suitable substituted alkyls which include carboxymethyl, carboxyethyl, carboxypropyl, carboxybutyl, carboxypentyl and carboxyhexyl.

Preferred compounds include those wherein A₄ is

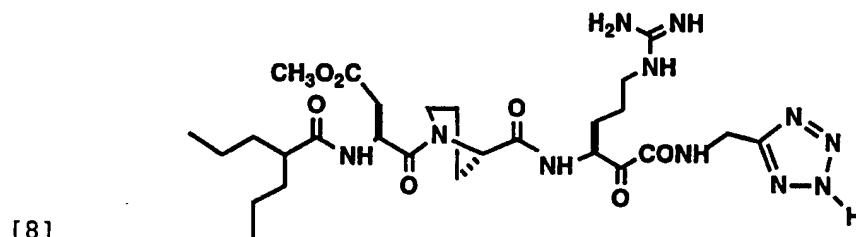
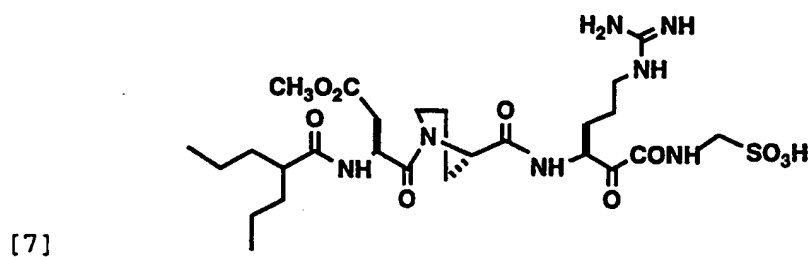
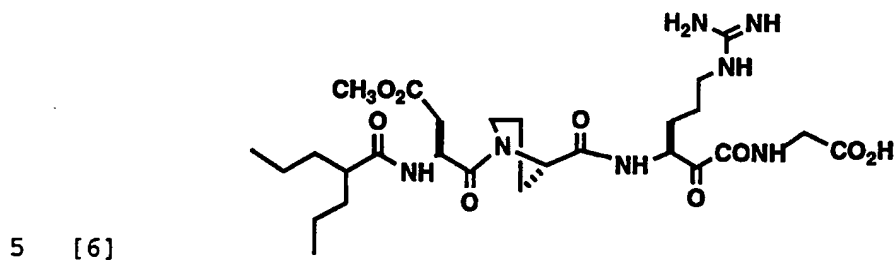
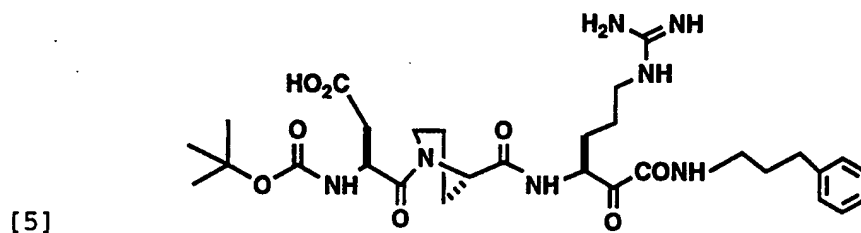
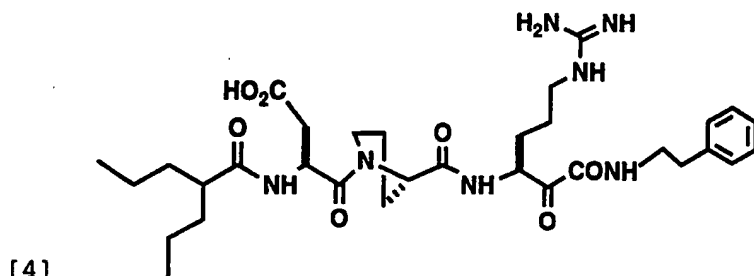


Especially preferred compounds include those
5 wherein R6, R7 or both are hydrogen, r is 0, and where
applicable R8 is benzyl or 2-phenylethyl.

Preferred compounds of the present invention include



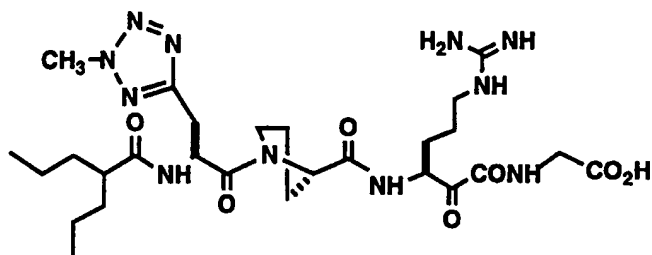
36



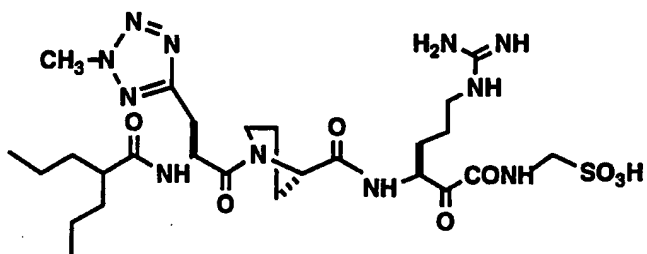
10

37

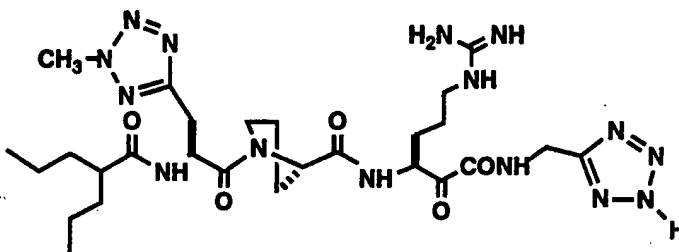
[9]



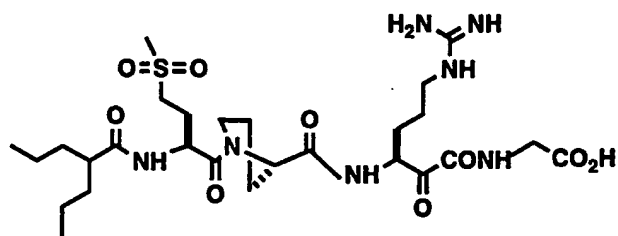
[10]



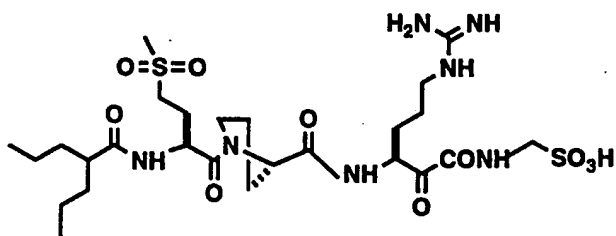
5 [11]



[12]



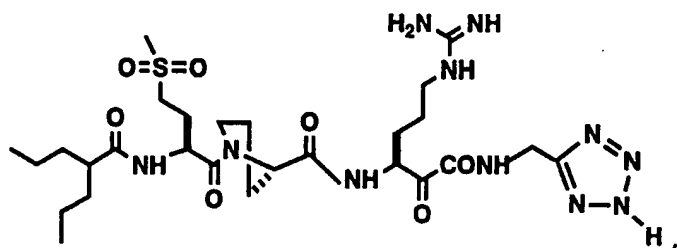
[13]



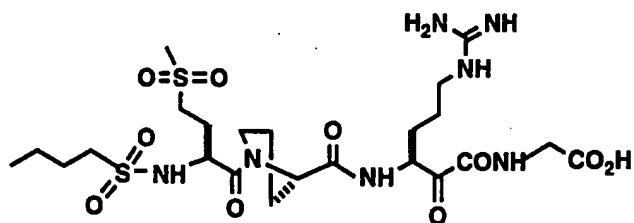
10

38

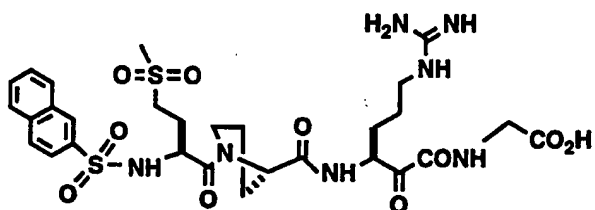
[14]



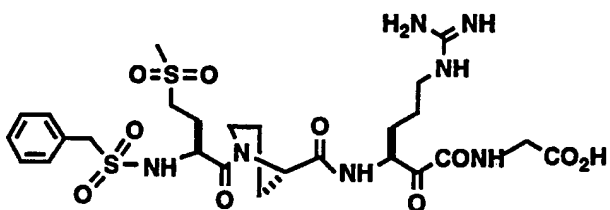
[15]



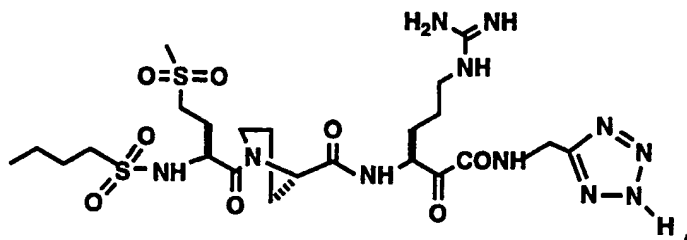
5 [16]



[17]

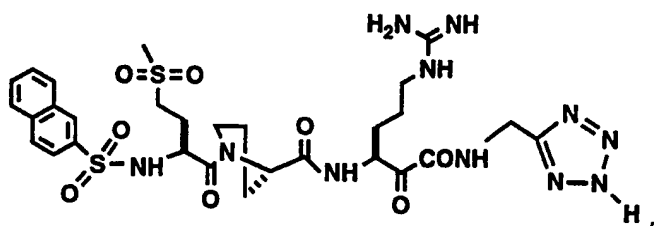


[18]



10

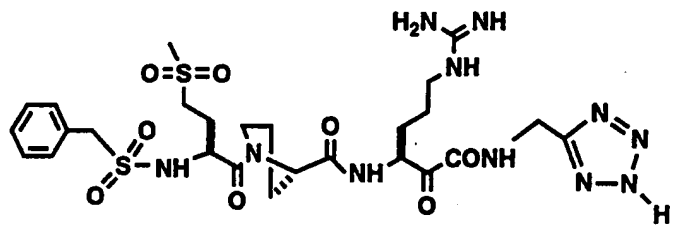
[19]



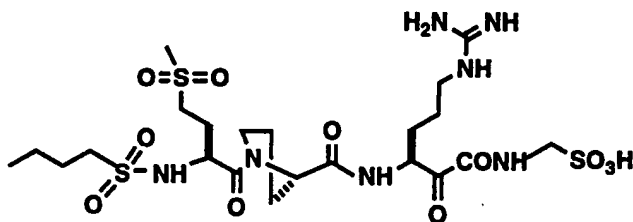
SUBSTITUTE SHEET

39

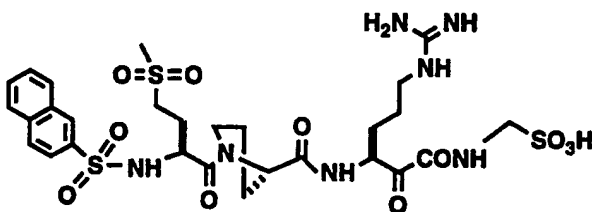
[20]



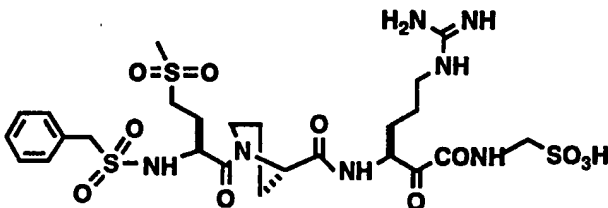
[21]



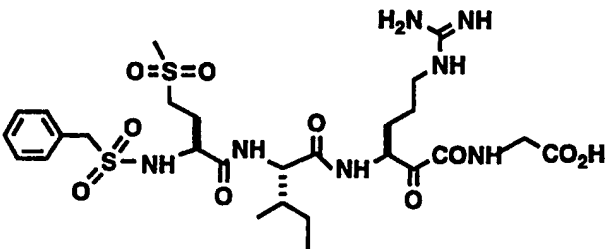
5 [22]



[23]

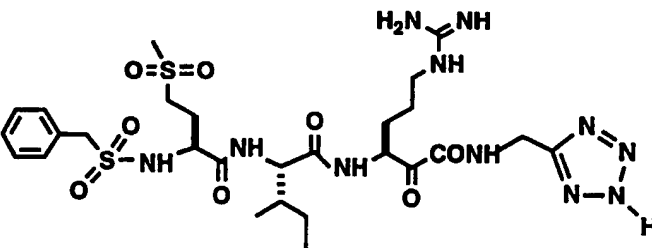


[24]



10

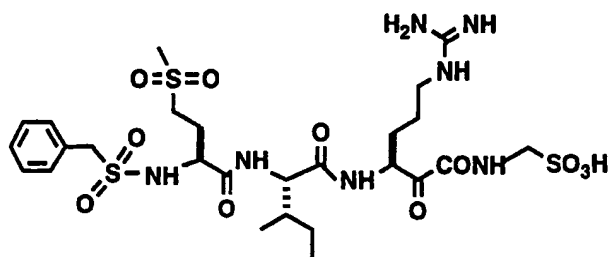
[25]



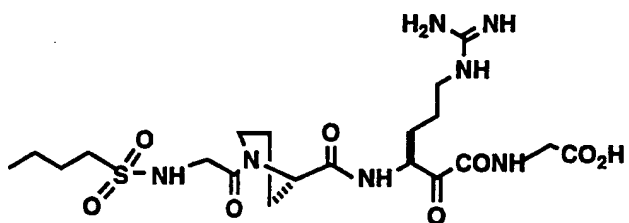
SUBSTITUTE SHEET

40

[26]

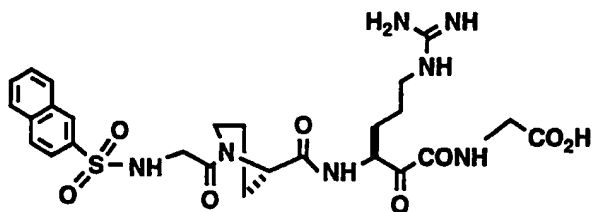


[27]

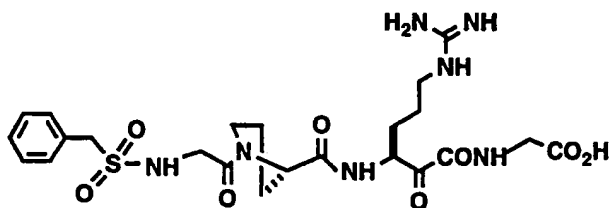


5

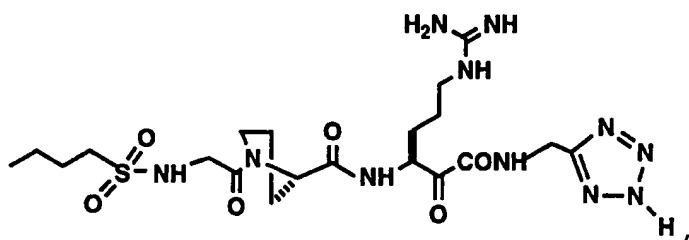
[28]



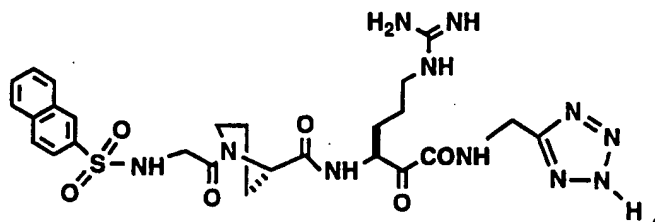
[29]



10 [30]



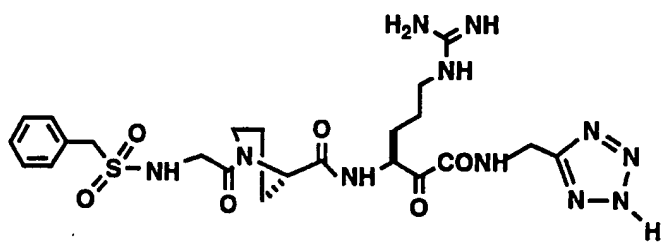
[31]



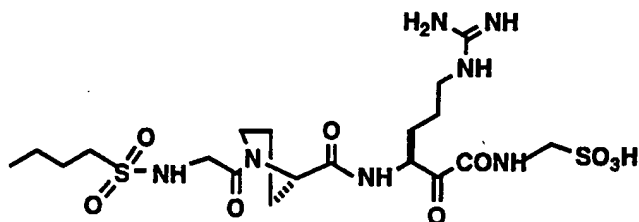
SUBSTITUTE SHEET

41

[32]

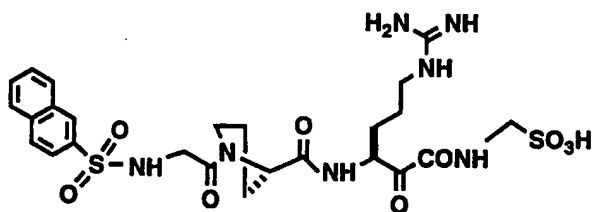


[33]

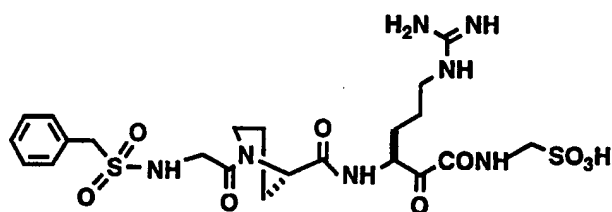


5

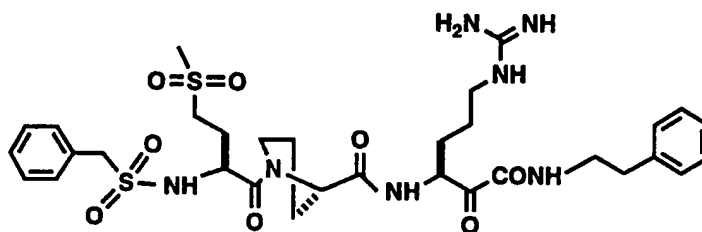
[34]



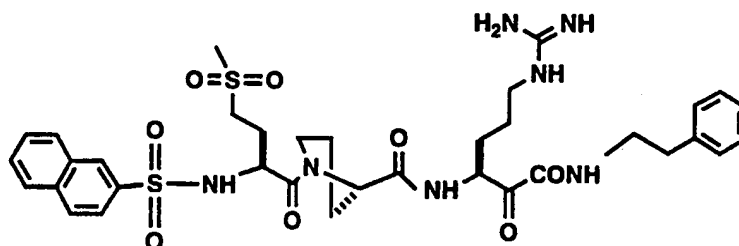
[35]



10 [36]

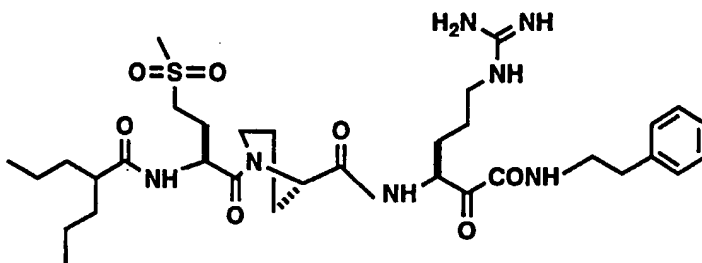
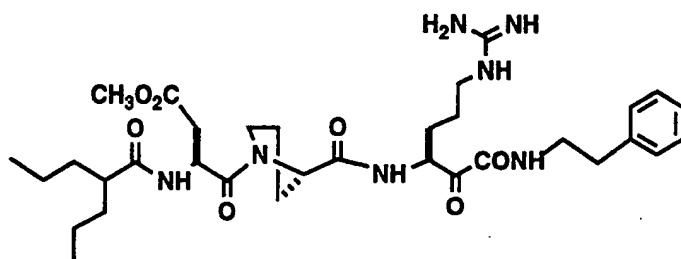


[37]

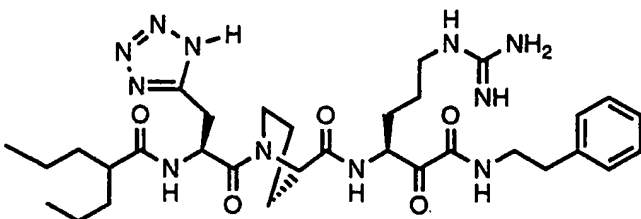
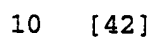
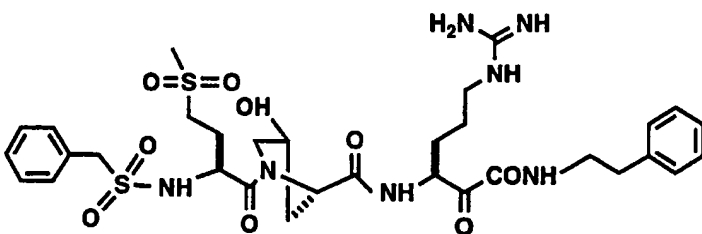
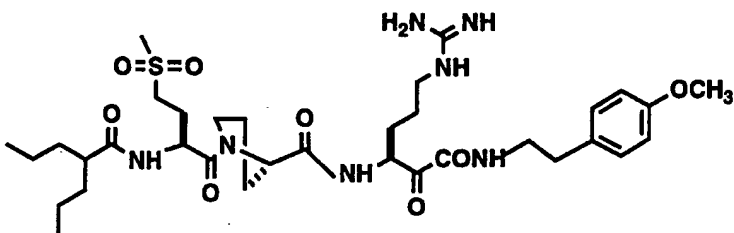


SUBSTITUTE SHEET

42



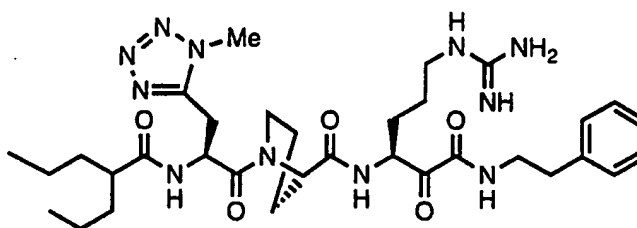
5



and

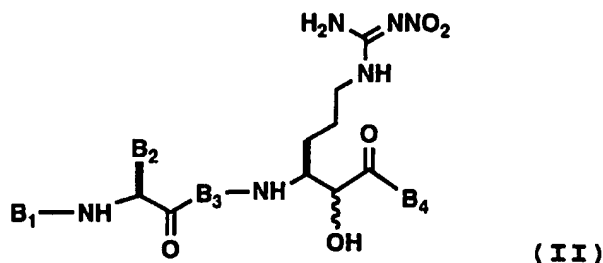
43

[43]



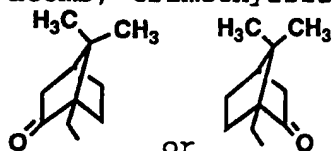
According to another aspect, the present invention is directed to pharmaceutically acceptable salts of the compounds of formula I. "Pharmaceutically acceptable salt" includes within its definition salts of the compounds of the present invention derived from the combination of a such compounds and an organic or inorganic acid. In practice, the use of the salt form amounts to use of the base form. The compounds of the present invention are useful in both free base and salt form, with both forms being considered as being within the scope of the present invention. These salts include acid addition salts, for example, salts of hydrochloric acid, hydrobromic acid, acetic acid, benzene sulfonic acid and other suitable acid addition salts.

In yet another aspect, the present invention is directed to compounds useful as intermediates for the preparation of compounds represented by formula I. These intermediates are represented by formula II:



The compounds of the present invention include those wherein B₁ is R₉-C(O)-, R₉-O-C(O)-, R₉-NH-C(O)-, R₉-S(O₂)-, R₉-O-S(O₂)- or R₉-NH-S(O₂)-. Preferred compounds include those wherein B₁ is R₉-C(O)-, R₉-O-C(O)- or R₉-S(O₂)-.

The compounds of the present invention include those wherein is alkyl of 1 to about 12 carbon atoms; alkenyl of about 3 to about 6 carbon atoms; aryl of about 6 to about 14 carbon atoms which is optionally
 5 mono-substituted with X₅ or optionally di-substituted with X₅ and X₆; aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X₅ or optionally di-substituted with X₅ and X₆; aralkenyl of about 8 to about 15 carbon atoms which is optionally
 10 mono-substituted with X₅ or optionally di-substituted with X₅ and X₆; perfluoroalkyl of 1 to about 12 carbon atoms; perfluoroaryl of about 6 to about 14 carbon atoms; trimethylsilylalkyl of 4 to about 8 carbon atoms,



or , wherein X₅ and X₆ are each indepen-
 15 dently selected independently and are bromo, chloro, fluoro, Y₃-, Y₃O-, Y₃O-C(O)-NH-, Y₃O-C(O)-N(Y₄)-, (Y₃,Y₄)N-, Y₃-C(O)-NH-, Y₃-S-, Y₃-S(O)-, Y₃-S(O₂)-, Y₃-O-S(O₂)-, NH₂-S(O₂)- or Y₃-NH-S(O₂)-, wherein Y₃ and Y₄ are independently selected and are trifluoromethyl,
 20 pentafluoroethyl, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms and alkyl of 1 carbon atom to about 12 which is optionally mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms.

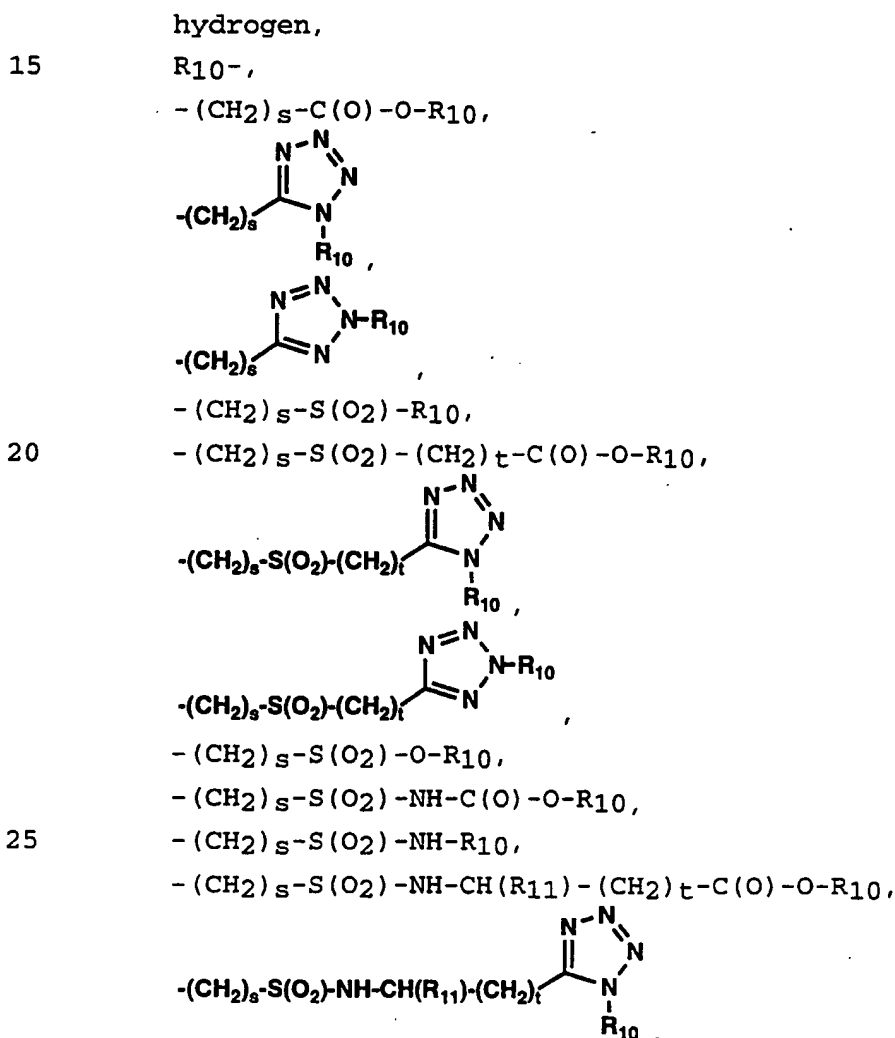
25 Preferred compounds include those wherein R₉ is alkyl of 1 to about 12 carbon atoms; aryl of about 6 to about 14 carbon atoms which is optionally mono-substituted with X₅ or optionally di-substituted with X₅ and X₆; or aralkyl of about 6 to about 15 carbon atoms which
 30 is optionally mono-substituted with X₅ or optionally di-substituted with X₅ and X₆.

Suitable alkyl groups include methyl, ethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, 2,2-dimethylpropyl, butyl, 3-methylbutyl, 1-propylbutyl, pentyl,

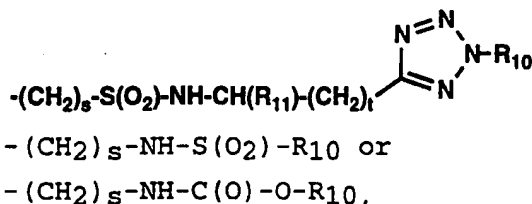
hexyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, adamantyl and adamantylmethyl. Suitable aryl groups include phenyl, naphthyl, biphenyl, pyridyl, 2-thienyl, 2-pyrrolyl and 2-furyl. Suitable
 5 aralkyl groups include phenylmethyl, diphenylmethyl, biphenyl, biphenylmethyl, naphthyl, naphthylmethyl, α -phenylmethylphenyl and 2-phenylethylene.

Especially preferred compounds include those wherein R₉ is 1,1-dimethylethyl, 2,2-dimethylpropyl,
 10 butyl, 3-methylbutyl, 1-propylbutyl, phenylmethyl or naphthyl.

The compounds of the present invention include those wherein B₂ is



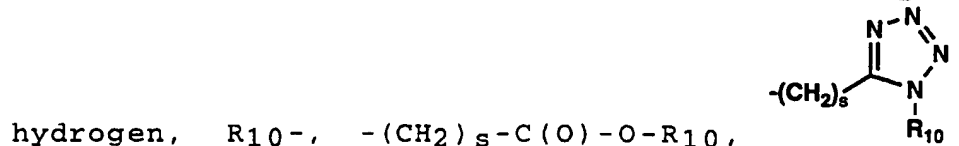
46

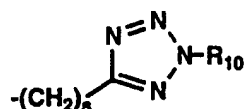


wherein s is 1, 2 or 3; t is 0, 1, 2, 3 or 4; R_{10} is
 5 alkenyl of about 3 to about 6 carbon atoms, aryl of
 about 6 to about 14 carbon atoms, aralkyl of about 6 to
 about 15 carbon atoms, aralkenyl of about 8 to about 15
 carbons atoms, or alkyl of 1 to about 12 carbon atoms
 which is optionally mono-substituted with aralkyloxy of
 10 about 6 to about 15 carbon atoms; and R_{11} is hydrogen,
 alkyl of 1 to about 4 carbon atoms, aryl of about 6 to
 about 14 carbon atoms, aralkyl of about 6 to about 15
 carbon atoms, or alkyl of 1 to about 4 carbon atoms sub-
 stituted with a substituent selected from the group con-
 15 sisting of -O-R_{10} , -C(O)-O-R_{10} , -C(O)-NH_2 , -S-CH_3 ,
 -S(O)-CH_3 , $\text{-S(O}_2\text{)-CH}_3$ and $\text{-NH-S(O}_2\text{)-CH}_3$.

Suitable alkyl groups include methyl, ethyl, 1,1-
 dimethylethyl, propyl, 2-methylpropyl, 2,2-dimethyl-
 propyl, butyl, 3-methylbutyl, 1-propylbutyl, pentyl,
 20 hexyl, cyclopentyl, cyclopentylmethyl, cyclohexyl,
 cyclohexylmethyl, adamantyl and adamantylmethyl.
 Suitable alkenyl groups include 2-propenyl, 3-butenyl,
 1-pentenyl, 2-pentenyl, 5-hexenyl and 2-cyclopentenyl.
 Suitable aryl groups include phenyl, naphthyl, biphenyl,
 25 pyridyl, 2-thienyl, 2-pyrrolyl and 2-furyl. Suitable
 aralkyl groups include phenylmethyl, diphenylmethyl,
 biphenyl, biphenylmethyl, naphthyl, naphthylmethyl, α -
 phenylmethylphenyl and 2-phenylethylene. Suitable aral-
 kyloxy groups include benzyloxymethyl.

30 Preferred compounds include those wherein B_2 is





or $-(CH_2)_s-S(O_2)-R_{10}$, wherein s is 1 or 2 and R_{10} is alkyl of 1 to about 12 carbon atoms which is optionally mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms.

5 Especially preferred compounds include those wherein s is 1 and R_{10} is methyl or benzyloxymethyl.

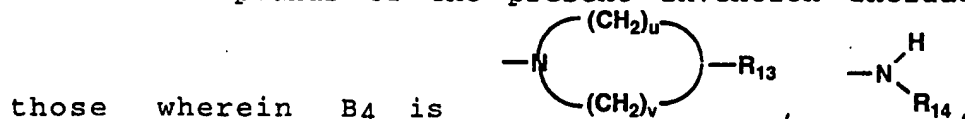
The compounds of the present invention include those wherein B_3 is an amino acid residue of L-alanine, L-azetidinecarboxylic acid, glycine, L-isoleucine, L-leucine, L-lysine mono-substituted at its ϵ -amino group with $R_2-S(O_2)-$, L-methionine sulfone, N-methylglycine, L-ornithine mono-substituted at its δ -amino group with $R_2-S(O_2)-$, L-pipecolic acid, L-phenylalanine, L-proline, L-valine, and trans-4-hydroxy-L-proline substituted at 10 4-hydroxy group with $R_{12}-O-C(O)-$, wherein R_{12} is alkyl of 1 to about 12 carbon atoms or aralkyl of about 6 to about 15 carbon atoms.

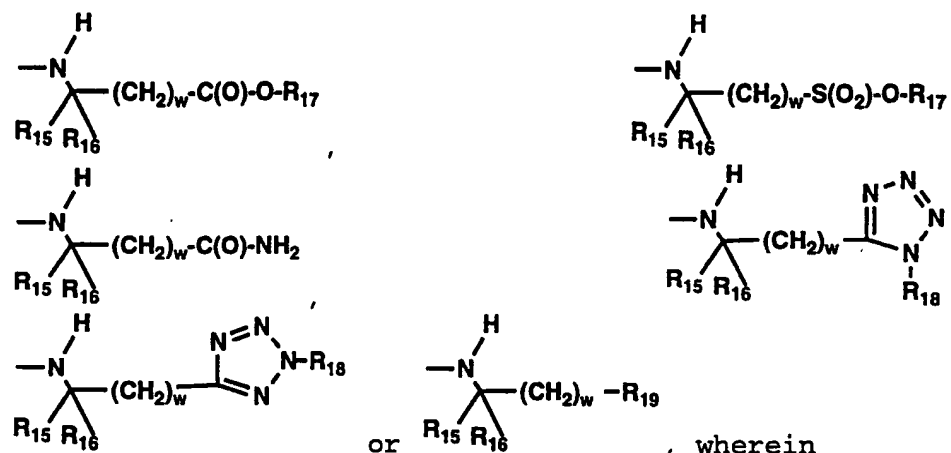
Suitable alkyl groups include methyl, ethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, 2,2-dimethylpropyl, butyl, 3-methylbutyl, 1-propylbutyl, pentyl, 20 hexyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, adamantyl and adamantylmethyl. Suitable aralkyl groups include phenylmethyl, diphenylmethyl, biphenyl, biphenylmethyl, naphthyl, naphthylmethyl, α -phenylmethylphenyl and 2-phenylethylene.

25 Preferred compounds include those wherein B_3 is glycine, L-isoleucine or proline.

Especially preferred compounds include those wherein B_3 is proline.

30 The compounds of the present invention include





u and v are each independently selected integers
5 from 1 to 5, where the sum of $u + v$ is 4 to 8;

R₁₃ is aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, alkoxy of 1 to about 4 carbon atoms, -NH-C(O)-O-X₇, -C(O)-O-X₇, -C(O)-NH₂, fluoro, -O-X₇, -NO₂ and -CF₃;

R₁₄ is aryl of about 6 to about 14 carbon atoms;

R₁₅ is hydrogen or alkyl of 1 to about 4 carbon atoms:

15 R16 is

hydrogen;

alkyl of 1 to about 4 carbon atoms:

aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH-C(O)-O-X₈, -C(O)-O-X₈, -C(O)-NH₂, fluoro, -O-X₈, -NO₂, -CF₃; alkyl of 1 to about 4 carbon atoms and alkoxy of 1 to about 4 carbon atoms;

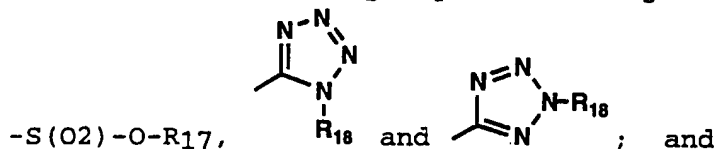
aralkyl of about 6 to about 15 carbon atoms
25 which is optionally substituted with 1 or 2 substituents
each independently selected from the group consisting of
-NH-C(O)-O-X₉, -C(O)-O-X₉, -C(O)-NH₂, fluoro, -O-X₉,
-NO₂, -CF₃, alkyl of 1 to about 4 carbon atoms, and
alkoxy of 1 to about 4 carbon atoms; and

alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -O-X₁₀, -C(O)-O-X₁₀, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and -NH-S(O₂)-CH₃; wherein X₇, X₈, X₉ and
 5 X₁₀ are independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms and aralkyl of about 6 to about 15 carbon atoms;

R₁₇ is alkyl of 1 to about 4 carbon atoms or
 10 aralkyl of about 6 to 15 carbon atoms;

R₁₈ is alkyl of 1 to about 12 carbon atoms which is optionally mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms;

R₁₉ is hydrogen; aryl of about 6 to about 14 carbon
 15 atoms which is optionally mono-substituted with X₁₁ or optionally di-substituted with X₁₁ and X₁₂; or aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X₁₁ or optionally di-substituted with X₁₁ and X₁₂; wherein X₁₁ and X₁₂ are independently
 20 selected from the group consisting of -C(O)-O-R₁₇,

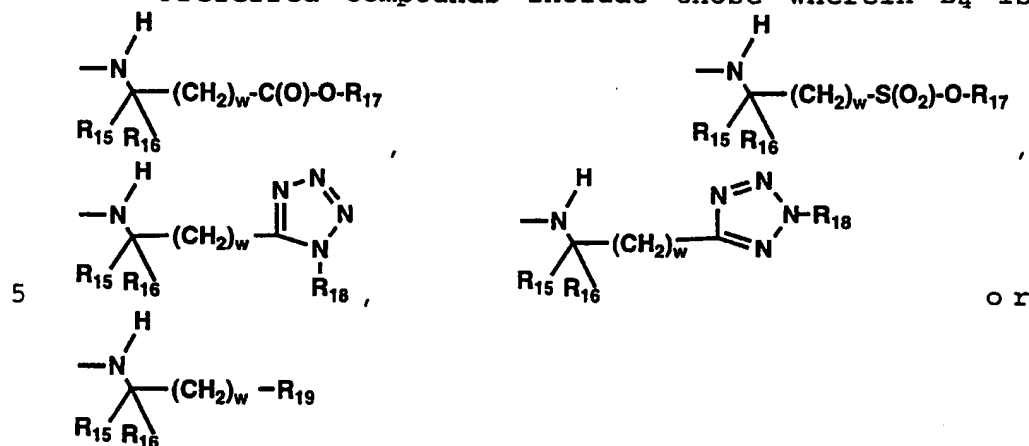


w is 0, 1, 2, 3, 4 or 5.

Suitable alkyl groups include methyl, ethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, 2,2-dimethyl-
 25 propyl, butyl, 3-methylbutyl, 1-propylbutyl, pentyl, hexyl, cyclopentyl, cyclopentylmethyl, cyclohexyl, cyclohexylmethyl, adamantyl and adamantylmethyl. Suitable aryl groups include phenyl, naphthyl, biphenyl, pyridyl, 2-thienyl, 2-pyrrolyl and 2-furyl. Suitable
 30 aralkyl groups include phenylmethyl, diphenylmethyl, biphenyl, biphenylmethyl, naphthyl, naphthylmethyl, α-phenylmethylphenyl and 2-phenylethylene. Suitable alkoxy groups include methoxy, ethyloxy, propyloxy,

butyloxy, isobutyloxy and pentyloxy, hexyloxy. Suitable aralkyloxy groups include benzyloxymethyl.

Preferred compounds include those wherein B₄ is



Especially preferred compounds include those wherein R₁₅, R₁₆ or both are hydrogen, w is 0, and where applicable R₁₇ is benzyl, R₁₈ is benzyloxymethyl or methyl, and R₁₉ is benzyl or 2-phenylethyl.

Preparation of Preferred Compounds

The preferred compounds of formula I may be conveniently prepared by liquid phase methods.

One method of synthesizing the compounds of formula I comprises converting the α-amino protected amino acid to an "activated" derivative wherein its carboxyl group is rendered more susceptible to reaction with the free N-terminal α-amino group of the target amino acid or peptide having an associated α-keto amide functionality.

For example, the free carboxyl of the α-amino protected (N-protected) amino acid can be converted to a mixed anhydride by reaction of a N-protected amino acid with ethyl chloroformate, pivaloyl chloride or like acid chlorides. Alternatively, the carboxyl of the α-amino protected amino acid can be converted to an active ester such as a 2,4,5-trichlorophenyl ester, a pentachlorophenol ester, a pentafluorophenyl ester, a p-nitrophenyl ester, a N-hydroxysuccinimide ester, or an ester formed from 1-hydroxybenzotriazole.

Another coupling method involves use of a suitable coupling agent such as N,N'-dicyclohexylcarbodiimide or N,N'-diisopropyl-carbodiimide. Other appropriate coupling agents are disclosed in E. Gross & J. Meinenhofer, *The Peptides: Analysis, Structure, Biology*, Vol. I: Major Methods of Peptide Bond Formation (Academic Press, New York, 1979).

The α -amino group of the target amino acid or peptide having an associated α -keto amide functionality employed in the synthesis of the compounds of the present invention is selectively de-protected during the coupling reaction to prevent side reactions involving the reactive, unprotected, side chain functionalities. In addition, reactive side-chain functional groups (e.g., amino, carboxyl, guanidinyll, hydroxyl, and sulfhydryl) must also be protected with suitable protecting groups to prevent chemical reaction of those groups from occurring during both the initial and subsequent coupling steps. Suitable protecting groups, known in the art, are described in E. Gross & J. Meienhofer, *The Peptides: Analysis, Structure, Biology*, Vol. 3: Protection of Functional Groups in Peptide Synthesis (Academic Press, New York, 1981).

In selecting suitable α -amino and reactive side-chain protecting groups to be used during synthesis of the compounds of formula I, the following considerations may be determinative. An α -amino protecting group should: (a) render the α -amino function inert (i.e., non-reactive) under the conditions employed in the coupling reaction, (b) be readily removable after the coupling reaction under conditions that will not remove side-chain protecting groups and will not alter the structure of the peptide fragment, and (c) minimize or eliminate the possibility of racemization upon activation immediately prior to coupling. An amino acid side-chain protecting group should: (a) render the protected side chain functional group inert under the conditions

employed in the coupling reaction, (b) be stable under the conditions employed in removing the α -amino protecting group, and (c) be readily removable upon completion of the desired peptide under reaction conditions that will not alter the structure of the peptide chain.

It will be apparent to those skilled in the art that the protecting groups known to be useful for liquid phase peptide synthesis may vary in reactivity with the agents employed for their removal. For example, certain protecting groups such as triphenylmethyl and 2-(p-biphenyl)isopropoxyloxycarbonyl are very labile and can be cleaved under mild acid conditions. Other protecting groups, such as t-butyloxycarbonyl, t-amylloxycarbonyl, adamantyl-oxycarbonyl, and p-methoxybenzyloxycarbonyl are less labile and require moderately strong acids, such as trifluoroacetic, hydrochloric, or boron trifluoride in acetic acid, for their removal. Still other protecting groups, such as benzyloxycarbonyl, halobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl cycloalkyloxycarbonyl, and isopropoxyloxycarbonyl, are even less labile and require stronger acids, such as hydrogen fluoride, hydrogen bromide, or boron trifluoroacetate in trifluoroacetic acid, for their removal.

Examples of amino acid protecting groups which are conventionally used include the following:

- (1) For an α -amino group: (a) aromatic urethane-type protecting groups, such as fluorenylmethyloxycarbonyl (Fmoc); (b) aliphatic urethane-type protecting groups, such as t-butyloxycarbonyl, t-amylloxycarbonyl, isopropoxyloxycarbonyl, 2-(p-biphenyl)-isopropoxyloxycarbonyl, allyloxycarbonyl and the like; (c) cycloalkyl urethane-type protecting groups, such as cyclo-pentyloxycarbonyl, adamantyloxycarbonyl, and cyclohexyloxy-carbonyl; and (d) allyloxycarbonyl. Preferred α -amino protecting

groups include t-butyloxycarbonyl or fluorenylmethyloxycarbonyl.

(2) For the side chain amino group present in lysine: protecting groups include any of the groups mentioned above in (1) such as t-butyloxycarbonyl, p-chlorobenzyloxycarbonyl, etc.

(3) For the guanidino group of arginine: protecting groups preferably include nitro, carbobenzyloxy, or 2,2,5,7,8-pentamethylchroman-6-sulfonyl or 2,3,6-trimethyl-4-methoxyphenylsulfonyl.

(4) For the hydroxyl group of serine, threonine, or tyrosine: protecting groups include, for example, t-butyl; benzyl; substituted benzyl groups, such as p-methoxybenzyl, p-nitrobenzyl, p-chlorobenzy, o-chlorobenzy, and 2,6-dichlorobenzy.

(5) For the carboxyl group of aspartic acid or glutamic acid: protecting groups include, for example, by esterification using groups such as t-butyl, indan-5-yl or preferably benzyl.

(6) For the imidazole nitrogen of histidine: suitable protecting groups include the benzyloxymethyl group.

(7) For the phenolic hydroxyl group of tyrosine: protecting groups such as tetrahydropyranyl, tert-butyl, trityl, benzyl, chlorobenzy, 4-bromobenzy, and 2,6-dichlorobenzy are suitably employed. The preferred protecting group is bromo-benzyloxycarbonyl.

(8) For the side chain sulfhydryl group of cysteine: trityl is preferably employed as a protecting group.

Starting materials used in the preparation of these compounds are readily available from commercial sources as Aldrich, Bachem BioScience Inc., Nova Biochemicals, and Sigma.

According to one suitable reaction scheme, the compounds of formula I are prepared according to the following protocol. The α -amino protecting group is

removed from a t-butyloxycarbonyl-protected amino acid or peptide having an associated α -keto amide functionality, such as by using trifluoroacetic acid in methylene chloride or trifluoroacetic acid alone. The deprotection is carried out at a temperature of from about 0°C to about ambient temperature. Other suitable cleaving reagents, for removal of specific α -amino protecting groups, such as HCl in dioxane, may be used.

After the α -amino protecting group is removed from the amino acid or peptide, the desired α -amino and side-chain protected amino acid is coupled to the α -amino deprotected amino acid or peptide. Additional α -amino and side chain protected amino acids are coupled in a stepwise manner in the desired order until the desired sequence has been completed. As an alternative to adding each amino acid separately during the synthesis, several amino acids may be coupled to one another to give a peptide fragment prior to their coupling to the target amino acid analog. After the coupling steps are complete, the product peptide analog is deprotected to give the compound of formula I. Selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable coupling reagents include N,N'-dicyclohexylcarbodiimide, diisopropylcarbodiimide or BOP.

The compounds of the present invention, represented in formula I above, are synthesized by a preferred liquid phase method as depicted in Figure 1 and described below using the intermediates represented by formula II above. Step A: The aldehyde functionality of the protected arginine aldehyde 1 is chemically replaced with an α -hydroxyacetic acid group to give the protected α -hydroxycarboxylic acid analogue of arginine, 6. Examples 2 to 4 describe a series of reactions exemplifying how the aldehyde group may be converted to an α -hydroxyacetic acid group.

Step B: The newly-introduced carboxy group on intermediate 6 is coupled using BOP to any suitable substituted amine, exemplified by amines such as 2-phenylethylamine in Example 5 or 3-phenylpropylamine as in Example 18, or by a protected amino acid as in Example 25 to give the amide 7. A suitable amine will be any sufficiently reactive amine in which other reactive groups are protected.

Step C: Suitably protected amino acids or peptides or peptide analogs are coupled to 7 after its N-terminus is deprotected. Examples 6, 9, 14 and 16 describe reactions coupling various N-protected-aspartyl-(β -benzyl ester)-prolyl derivatives to amide 7, to give derivatives containing the α -hydroxycarboxylic analogues of arginine, as exemplified by Examples 6, 9, 12, 15, and 19.

Step D: The α -hydroxygroup of the resulting derivatives is oxidized to a keto group under modified Moffatt conditions. Edwards et al., J. Am. Chem. Soc., 114: 1854 at 1861 (1992). This gives the corresponding α -ketoamide derivatives, which are examples of the intermediates of the present invention. Exemplars of these intermediates are the compounds of Examples 7, 10, 13, 16 and 20.

Step E: The protecting groups on the α -ketoamide derivatives is removed by means of catalytic hydrogenation (H_2 /Pd on Carbon) or HF deprotection using HF/anisole to give the compounds of the present invention. Exemplars of these compounds are the compounds of Examples 8, 11, 14, 17 and 21.

Purification of the compounds of the present invention is typically achieved using conventional procedures such as preparative HPLC (including reversed phase HPLC) or other known chromatography, affinity chromatography (including monoclonal antibody columns) or counter-current distribution.

Utility and Formulation

The present invention provides the novel compounds of formula I, their pharmaceutically acceptable salts and compositions prepared from them. These compounds and pharmaceutical compositions are useful as inhibitors of coagulation proteases, both in vitro and in vivo. As discussed in the Background and Introduction to the Invention, the formation of thrombin catalyzed by factor Xa is the penultimate reaction in the coagulation cascade and is a reaction common to both the intrinsic and extrinsic coagulation pathways which terminate in the formation of a fibrin clot. Inhibitors of this and other activated coagulation factors would therefore inhibit fibrin deposition, thrombus formation and the consumption of coagulation proteins.

Inhibitors of activated coagulation proteases may be used as pharmacological agents for the treatment of thrombotic disorders including, myocardial infarction, unstable angina, disseminated intravascular coagulation and associated complications resulting from venous thrombosis. These inhibitors may be used as adjunctive or conjunctive agents to prevent recurrent thrombosis following enzymatic thrombolysis and percutaneous transluminal angioplasty. In addition, specific inhibitors of factor Xa may be useful in the suppression of metastatic migration of certain tumor types as described by Tuszynski, G. P. et. al., "Isolation and characterization of antistasin, an inhibitor of metastasis and coagulation", J. Biol. Chem., 262: 9718-9723 (1987) and Brankamp, R. G. et. al., "Ghilantens: anticoagulants, antimetastatic proteins from the South American leech *Haementeria ghilianii*", J. Lab Clin. Med., 115: 89-97 (1990).

In mammals, in vivo uses would include administration of these compounds and compositions as therapeutic agents to prevent the formation of fibrin clots in blood vessels resulting from the presence of activated coagu-

lation proteases, to prevent abnormal thrombus formation resulting from thrombotic disorders, and to prevent or treat the recurrent thrombus formation resulting from chemical or mechanical intervention directed to clearing
5 blocked vessels. Additionally, the compounds, their salts and various compositions derived therefrom may be useful as therapeutic agents for suppressing the metastatic migration of tumor types in mammals.

The in vitro inhibitory activity of the compounds
10 of the present invention may be demonstrated using an enzyme inhibition assay. The test compound is dissolved in a suitable assay buffer to give a solution having a concentration of test compound under assay conditions in the range of from 0 to about 100 mM. The enzyme whose
15 activity is to be assayed is added to a solution containing a specified concentration of the test compound. After an incubation period, synthetic substrate for the enzyme is added. The rate of substrate turnover is determined spectrophotometrically at particular sub-
20 strate concentrations. This data is used to determine an inhibition constant, K_i , for the test compound. Example A demonstrates that the compounds of Examples 8, 17, and 21 are potent inhibitors of human α -thrombin, having K_i 's of 11, 1.5 and 5.5 nanomolar, respectively.
25 These assay results demonstrate that the compounds of formula I are active as inhibitors of thrombin in vitro. These assays are also considered to be indicative of in vivo activity.

The in vivo inhibitory activity of the compounds of
30 formula I in a rat model of acute thrombosis was demonstrated. (See Example B). The test compound was dissolved in a suitable diluent to give a test solution. The test solution is injected into a rat and the antithrombotic effect was measured. Example B demon-
35 strated that the compound of Example 8 possessed antithrombic efficacy in vivo in a mammal. The com-

pounds of the present invention are useful as inhibitors of thrombus formation.

Thus, in one aspect, the present invention is directed to methods for preventing or treating a condition in mammals characterized by abnormal thrombus formation. The pharmaceutically effective amount of the compound or composition of the present invention required as a dose will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. The dose can be tailored to achieve optimal efficacy but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

In practicing the methods of the invention, the compounds or compositions of the present invention can be used alone or in combination with one another, or in combination with other therapeutic or diagnostic agents. These compounds can be used in vivo, ordinarily in a mammal, preferably in a human, or in vitro. In employing them in vivo, the compounds or compositions can be administered to the mammal in a variety of ways, including parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, employing a variety of dosage forms.

In another aspect the present invention is directed to pharmaceutical compositions prepared for storage and subsequent administration which comprise a therapeutically effective amount of a compound of formula I or its pharmaceutically acceptable salt in a pharmaceutically acceptable carrier or diluent.

The therapeutically effective amount of compound of formula I or its pharmaceutically acceptable salt which will be required as a dose will depend on factors which include the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. One skilled in the

medical art will appreciate that each composition of therapeutic drug has individual characteristics relating to drug absorption which may affect the amount to be compounded into a given pharmaceutically acceptable carrier or diluent for a therapeutically effective dose. 5 Ansel, H., "Dosage Forms and Routes of Administration", *Introduction to Pharmaceutical Dosage Forms*, 4th Edition, pp. 49-62, Lea & Febiger, Philadelphia (1985).

As will be readily apparent to one skilled in the art, the useful in vivo dosage to be administered and the particular mode of administration will vary depending upon the age, weight and mammalian species treated, the particular compounds employed, and the specific use for which these compounds are employed. The determination of effective dosage levels, that is the dosage levels necessary to achieve the desired result, will be within the ambit of one skilled in the art. Typically, applications of compound are commenced at lower dosage levels, with dosage level being increased until the 15 desired effect is achieved. 20

The dosage for the compounds of the present invention, their pharmaceutically acceptable salts when compounded into pharmaceutically acceptable carriers or diluents can range broadly depending upon the desired affects and the therapeutic indication. Typically, dosages will be between about 0.01 mg/kg and 100 mg/kg body weight, preferably between about 0.01 mg/kg and 10 mg/kg body weight. Administration is preferably parenteral, such as intravenous on a daily basis. 25

The compounds of formula I may be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions, suspensions for injectable administration; and the like. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. 30 35

Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride, and the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. Ansel, H., *Introduction to Pharmaceutical Dosage Forms*, 4th Edition, pp. 117-358, Lea & Febiger, Philadelphia (1985). Also, if desired, absorption enhancing preparations (e.g., liposomes) may be utilized.

Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985). Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid may be added as preservatives. Id. at 1449. In addition, antioxidants and suspending agents may be included. Id.

To assist in understanding the present invention, the following examples are included which describe the results of a series of experiments. The following examples relating to this invention should not, of course, be construed as specifically limiting the invention. Variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

Certain of the Examples, including Examples 1 to 29 illustrate the preparation of the compounds of the present invention according to the synthetic scheme depicted in Figure 1. Example A illustrates the activity and use of the compounds of the present invention as an inhibitor of thrombin. Example B illustrates the in

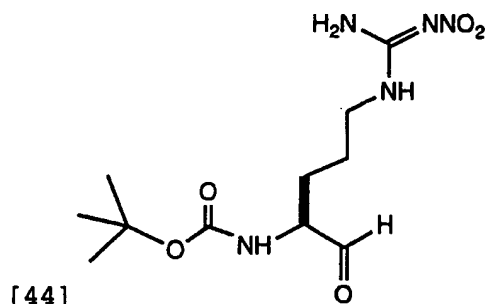
vivo activity use of a compound of the present invention in mammals as an antithrombotic agent.

EXAMPLES

5 Example 1

Preparation of

Alpha-N-t-butoxycarbonyl-N^g-nitroargininal



10

The following procedure for the synthesis of alpha-t-butoxycarbonyl-N^g-nitro-argininal (Compound of Example 1) is a modification of the procedure of Fehrentz, J.A. and Castro, B., Synthesis, 676 (1983).

15 BOC-N^g-nitroarginine was obtained from Calbiochem. N-methylpiperidine, N,O-dimethylhydroxylamine hydrochloride, isobutylchloroformate, and lithium aluminum hydride were obtained from Aldrich Chemical Company, Inc. Dichloromethane, ethyl acetate, methanol, and
20 tetrahydrofuran were obtained from Fisher Scientific Company.

11.4 mL of N-methylpiperidine was slowly added to a stirred suspension of 9.17 g (94 mmole) of N,O-dimethylhydroxylamine hydrochloride in 75 mL of dichloromethane
25 which had been cooled to about 0°C. The solution was allowed to stir for 20 minutes and was kept cold for use in the next step.

In a separate flask, 30.0 g (94 mmole) of Boc-N^g-nitroarginine was dissolved by heating in about 1400 mL
30 of tetrahydrofuran and cooled under nitrogen to 0°C. 11.4 mL of N-methylpiperidine and 12.14 mL (94 mmole) of

isobutylchloroformate were added and the mixture was stirred for 10 minutes. The free hydroxylamine solution prepared above was added in one portion and the reaction mixture was allowed stir overnight at room temperature.

5 The resulting precipitate was removed by filtration and washed with 200 mL of tetrahydrofuran. After concentrating the filtrates to about 150 mL under vacuum, 200 mL of ethyl acetate was added, followed by ice to cool the solution. The cooled solution was washed with
10 two 75 mL portions of 0.2 N hydrochloric acid, two 75 mL portions of 0.5 N sodium hydroxide, one 75 mL portion of brine, then was dried with anhydrous magnesium sulfate. Upon concentration under vacuum, 22.7 g (70% yield) of solid BOC-N⁹-nitroarginine N-methyl-O-methylcarboxamide
15 was isolated. Thin layer chromatographic analysis in 9:1 dichloromethane/methanol (silica gel) showed one spot.

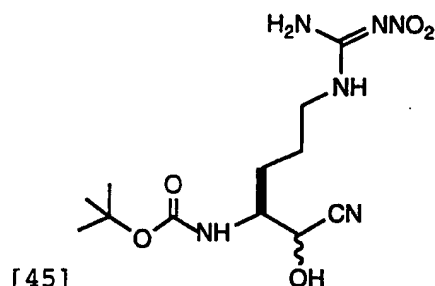
A flask was placed under a nitrogen atmosphere and cooled to -50°C, then was charged with 70 mL (70 mmole)
20 of 1 M lithium aluminum hydride (in tetrahydrofuran) and 500 mL of dry tetrahydrofuran. A solution containing 66 mmole of BOC-N⁹-nitroarginine N-methyl-O-methylcarboxamide in 50 mL of dry tetrahydrofuran was slowly added while the temperature of the reaction mixture was maintained at -50°C. After allowing the reaction mixture to
25 warm to 0°C by removal of the cooling bath, it was cooled to -30°C, at which temperature, 100 mL (0.2 mole) of 2 N potassium bisulfate was added with stirring, over a 10 to 15 minute period. The reaction mixture was then
30 allowed to stir at room temperature for 2 hours. After removal of the precipitate by filtration, the filtrate was concentrated to 100 mL under vacuum. The concentrate was combined with 200 mL ethyl acetate, then washed with two 50 mL portions of 1 N hydrochloric acid,
35 two 50 mL portions of saturated sodium bicarbonate, one 50 mL portion of brine, then was dried over anhydrous

magnesium sulfate. The mixture was concentrated under vacuum to yield 13.6 g (70%) of the title compound.

Example 2

5 Preparation of

N-(Nitroguanidino-1-(S)-(cyanohydroxymethyl)butyl)-1-(1,1-dimethylethoxy)methanamide



10

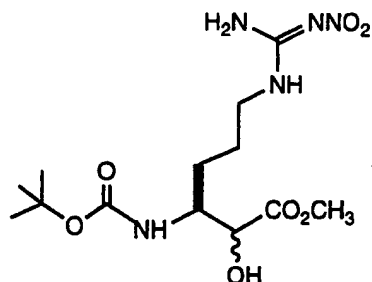
A solution of 25.2 g (83.1 mmols) of alpha-Boc-N⁹-nitro-argininal (the compound of Example 1) in 680 mL tetrahydrofuran was added to a solution of 136 g (1.36 moles) of potassium bicarbonate and 27.6 g (423 mmols) of potassium cyanide in 680 mL of water. This two phase mixture was allowed to stir vigorously for thirty minutes. The stirring was discontinued and the phases were separated. The aqueous phase was extracted three times with 500 mL ethyl acetate. The tetrahydrofuran phase was diluted with 1000 mL of ethyl acetate. The organic phases were combined and extracted successively with water and brine. This solution was dried over anhydrous magnesium sulfate and concentrated under vacuum to give 28.1 g of the above-identified product as a white foam.

25 This material can be purified by flash chromatography (0 to 6% methanol in dichloromethane) or carried through the next steps directly. ¹H NMR (CD₃OD) δ 1.37 (s, 9H), 1.53 (m, 2H), 1.7 (m, 2H), 3.19 (m, 2H), 3.65 (m, 1H), 4.29 (d, J=7 Hz, 0.35H), 4.48 (d, J=4 Hz, 0.65H).

30

Example 3Preparation of6-Nitroguanidino-3-(S)-(1,1-dimethylethoxy)methanamido-2-hydroxyhexanoic acid methyl ester

5



[46]

The 26.0 g (~83 mmole) crude cyanohydrin (compound of the Example 2) was dissolved in 450 mL dioxane, and 450 mL concentrated aqueous hydrochloric acid was slowly added with stirring. This addition was accompanied by vigorous gas evolution. This solution was heated to reflux and stirred for 15 hours. After this period of time, the reaction was allowed to cool to room temperature and then concentrated under vacuum to a thick brown syrup of 6-nitroguanidino-3-(S)-amino-2-hydroxyhexanoic acid hydrochloride salt (compound 3 of Figure 1).

Crude amino acid 3 (of Figure 1) from above was concentrated several times from methanol under vacuum and then dissolved in 750 mL of saturated anhydrous hydrochloric acid in methanol. This suspension was refluxed for three hours, allowed to cool to room temperature and concentrated under vacuum. This gave crude 6-nitroguanidino-3-(S)-amino-2-hydroxyhexanoic acid methyl ester hydrochloride salt (compound 4 of Figure 1) as a thick brown syrup. This was used directly in the next step.

The amino ester (compound 4 of Figure 1) from above was dissolved in a mixture of 300 mL of saturated sodium bicarbonate and 300 mL tetrahydrofuran. This mixture was

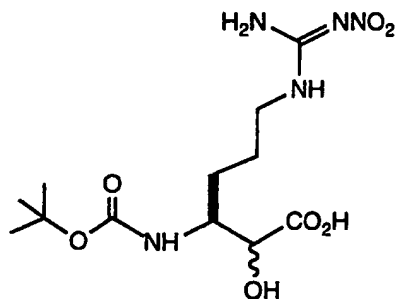
65

treated with di-t-butyldicarbonate (30 g, 137 mmol) and allowed to stir vigorously for 16 hours. The resulting mixture was extracted with ethyl acetate (1000 mL). The organic layer was washed successively with water then brine, dried over anhydrous magnesium sulfate and concentrated to a small volume under vacuum. The product was purified by flash chromatography (0 to 10% methanol/dichloromethane) to give 13.5 g (49% yield) of the above-identified product as an off-white foam. ¹H NMR (CDCl₃) δ 1.41 and 1.45 (s, 9H), 1.7 (m, 4H), 3.2 (m, 2H), 3.82 and 3.84 (s, 3H), 4.10 (m, 1H), 4.19 (bs, 0.65H), 4.33 (bs, 0.35H), 5.02 (d, J=10 Hz, 1H), 5.17 (d, J=10 Hz, 1H).

15 Example 4

Preparation of

6-Nitroguanidino-3-(S)-(1,1-dimethylethoxy)methanamido-2-hydroxyhexanoic acid



[47]

A solution of the compound of Example 3 (5.0 g, 13.8 mmol) in 100 mL of methanol was treated with 17 mL of 1 M lithium hydroxide. This solution was allowed to stir overnight and then treated with 20 mL of Dowex-50 resin X8 400 (H⁺ form) in 50 mL of deionized water. This solution was swirled for 15 minutes then passed through a 4 x 4 cm. column of the same resin, the column was washed with 1:1 methanol:water and the combined filtrates were concentrated to dryness under vacuum.

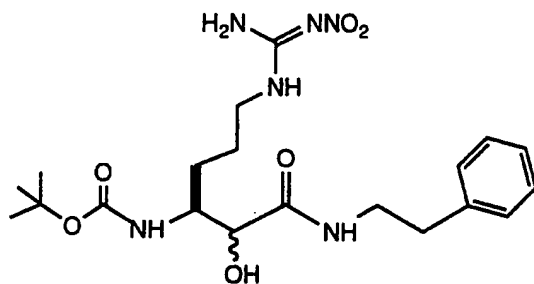
SUBSTITUTE SHEET

The residue was dissolved in 100 mL acetonitrile and concentrated to dryness, this process was repeated two more times to give 4.2 g (87 % yield) of the above-identified compound as an off-white foam. ^1H NMR (CD_3OD) δ

5 1.42 and 1.42 (s, 9H), 1.7 (m, 4H), 3.3 (m, 2H), 3.95 (m, 1H), 4.19 (bs, 0.65H), 4.33 (bs, 0.35H), 4.15 (d, $J=1$ Hz, 0.65H), 4.38 (d, $J=4$ Hz).

Example 5

10 Preparation of



[48]

15 A 1.05 g portion (2.90 mmole) of the compound of Example 3 was dissolved with stirring in 29 mL of methanol. To this solution was added 3.6 mL of 1N aqueous sodium hydroxide. After 18 hours, thin layer chromatographic analysis (10% methanol/dichloromethane)

20 showed no more starting material. The reaction was neutralized with 1.1 mL of 1N aqueous hydrochloric acid and concentrated under vacuum to dryness. The resulting solid was then dissolved in 15 mL of dimethylformamide with stirring. This solution was treated successively

25 with 0.364 mL (2.90 mmole) 2-phenylethylamine, 0.86 mL (7.83 mmole) NMM and 1.41 g (3.19 mmole) BOP. After 18 hours, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no more material corresponding to the acid. The reaction mixture was poured

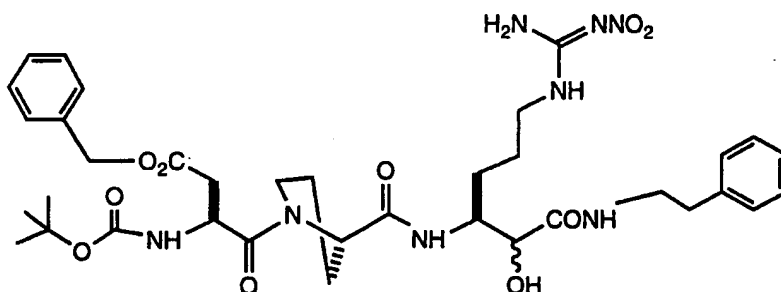
30 into ethyl acetate (300 mL) and washed successively with 1N aqueous hydrochloric acid (75 mL), water (75 mL),

saturated sodium bicarbonate (75 mL) and brine (75 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum to a foam. Flash chromatography (silica, 10% methanol/dichloromethane) afforded 1.18 g (90%) of a foam. $R_f = 0.33$ (two spots, 10% methanol/dichloromethane).

Example 6

Preparation of

10



[49]

To a solution of the compound of Example 5 (0.675 g, 1.49 mmole) in 17 mL dichloromethane was added 17 mL of trifluoroacetic acid with stirring. After 30 minutes, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no starting material. The trifluoroacetate salt was precipitated by adding 200 mL of diethyl ether and cooled in the freezer for 3 hours. The solid was removed by filtration and rinsed with 75 mL diethyl ether. The resulting solid was dissolved in 7 mL of dimethylformamide with stirring and this solution was treated with 0.627 g (1.49 mmole) α -N-(t-butoxycarbonyl)-L-aspartyl-(β -benzyl ester)-L-proline (the compound of Example 24), 0.44 mL (4.02 mmole) NMM and 0.72 g (1.64 mmole) BOP. After 18 hours, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no more trifluoroacetate salt. The mixture was added to 300 mL of ethyl acetate and washed successively with 75 mL of 1N aqueous hydrochloric acid, 75 mL of

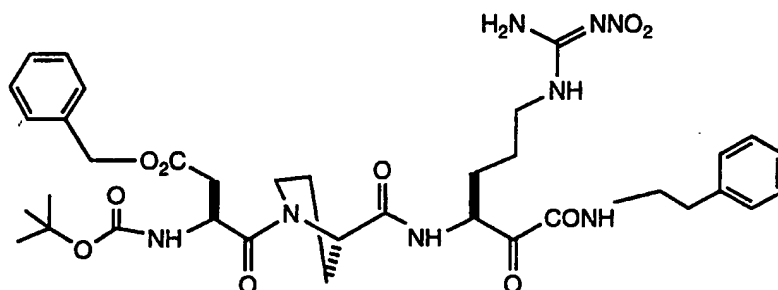
68

water, 75 mL of saturated sodium bicarbonate and 75 mL of brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum. Flash chromatography (silica, 3:1:9 hexane/methanol/dichloromethane) afforded 0.891 g (79%) of a foam. R_f = 0.29 (10% methanol/dichloromethane).

Example 7

Preparation of

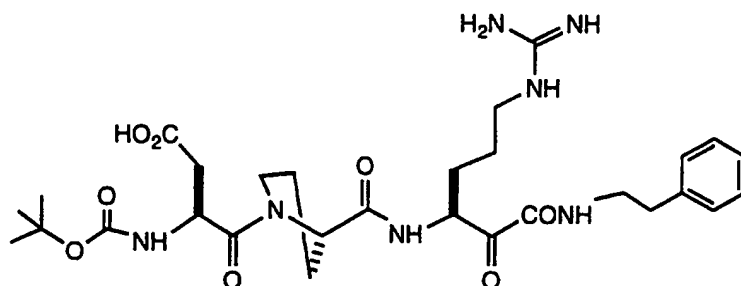
10



[50]

A 1.0 g portion (1.32 mmole) of the compound of Example 6 was dissolved in 13 mL of dimethylsulfoxide with stirring. This solution was treated with 13 mL of toluene and 2.53 g (13.23 mmole) of ethyl-3-(3-dimethylamino)-propylcarbodiimide hydrochloride followed by 0.43 mL (5.29 mmole) of dichloroacetic acid. After 1 hour, thin layer chromatographic analysis (10% methanol/dichloromethane) showed a new spot and no starting material. The mixture was added to 500 mL of ethyl acetate and washed with two 200 mL portions of water and 150 mL of brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum. Flash chromatography (silica, 4:1:4 hexane/methanol/dichloromethane) afforded 0.832 g (83%) of the above-identified compound as a foam. R_f = 0.32 (10% methanol/dichloromethane).

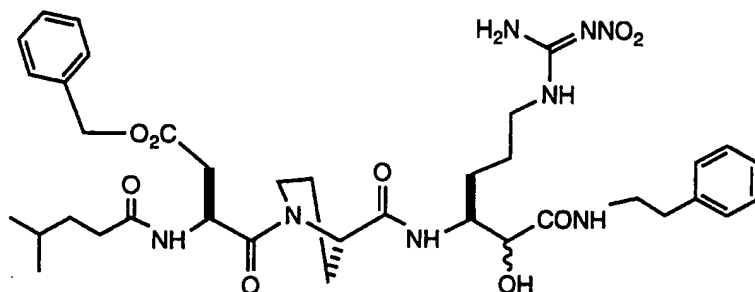
30

Example 8Preparation of

5

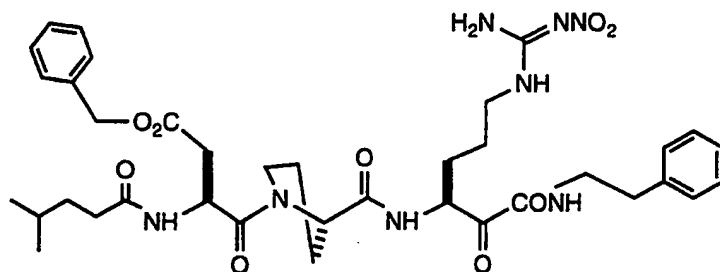
[3]

A 0.512 g portion (0.667 mmole) of the compound of Example 7 was dissolved in 50 mL of methanol. This solution was added to a Parr vessel containing 0.5 g 10% Pd/C, followed by 1.32 mL (1.32 mmole) 1N aqueous hydrochloric acid. The mixture was shaken under a 10 psig hydrogen atmosphere for 1.5 hours, after which HPLC (reverse phase, 1 mL/minute, 40-80% acetonitrile/water with 0.1% trifluoroacetic acid, 20 minute program, retention time = 6.09 minute) showed complete reaction. The mixture was filtered, rinsed with 10 mL of methanol and concentrated under vacuum. The resulting foam was purified by preparative HPLC (reverse phase, 50 mL/minute, 20-60% acetonitrile/water with 0.1% trifluoroacetic acid, 40 minute program). The appropriate fractions were combined and the acetonitrile was removed under vacuum. The remaining liquid was frozen and lyophilized to afford 0.25 g (36%) of the above-identified compound a white fluffy powder. Mass spectral analysis showed the expected molecular ion at 617.3 (calc. 617.3).

Example 9Preparation of

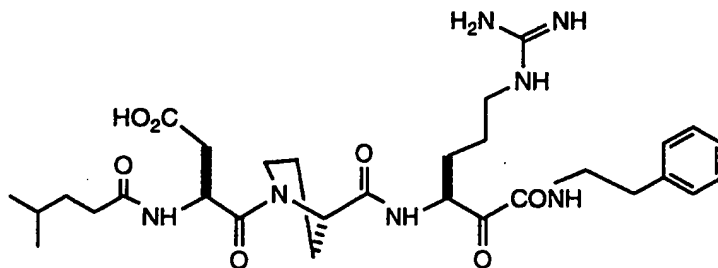
5 [51]

A solution of the compound of Example 6 (0.566 g, 0.749 mmole) in 14 mL of dichloromethane and 14 mL trifluoroacetic acid was allowed to stir at room temperature. After 40 minutes, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no starting material. The trifluoroacetate salt was precipitated by adding 200 mL diethyl ether. The mixture was allowed to cool in the freezer for 3 hours. The solid was removed by filtration, rinsed with 75 mL of diethyl ether, dissolved in 4 mL of dimethylformamide with stirring, and 0.062 mL (0.749 mmole) of 4-methylvaleric acid and 0.2 mL (2.02 mmole) of NMM were added, followed by 0.36 g of (0.82 mmole) of BOP. After 18 hours, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no more trifluoroacetate salt. The mixture was added to 300 mL of ethyl acetate and washed successively with 100 mL of 1N aqueous hydrochloric acid, 100 mL of water, 100 mL of saturated sodium bicarbonate and 100 mL of brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum to a foam. Flash chromatography (silica, 3:1:9 hexane/methanol/dichloromethane) afforded 0.317 g (56%) of the above compound as a foam. R_f = 0.32 (two spots, 10% methanol/dichloromethane).

Example 10Preparation of

[52]

A 0.236 g portion of the compound of Example 9 (0.313 mmole) was oxidized and worked up as described in Example 7. Flash chromatography (silica, 3:1:9 hexane/methanol/ dichloromethane) of the concentrated organic layer afforded 0.206 g (88%) of the above-identified compound as a foam. $R_f = 0.42$ (10% methanol/dichloromethane).

Example 11Preparation of

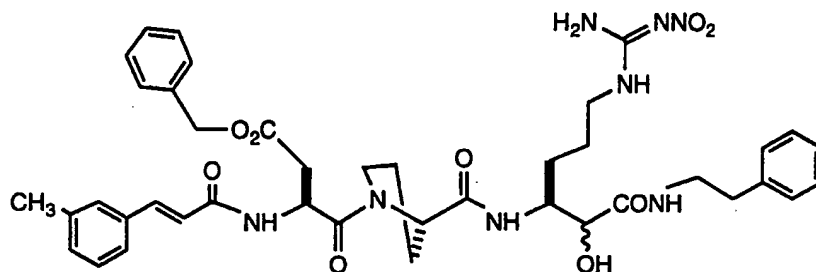
[2]

A 0.144 g portion of the compound of Example 10 (0.192 mmole) was hydrogenated and worked up as described in Example 8. The concentrate was purified by preparative HPLC (reverse phase, 50 mL/minute, 20-80% acetonitrile/water with 0.1% trifluoroacetic acid, 40 minute program). The appropriate fractions were combined and the acetonitrile was removed under vacuum.

72

The remaining liquid was frozen and lyophilized to afford 0.57 g (57%) the above-identified compound as a white fluffy powder. Mass spectral analysis showed the expected molecular ion at 615.3 (calc. 615.3).

5

Example 12Preparation of

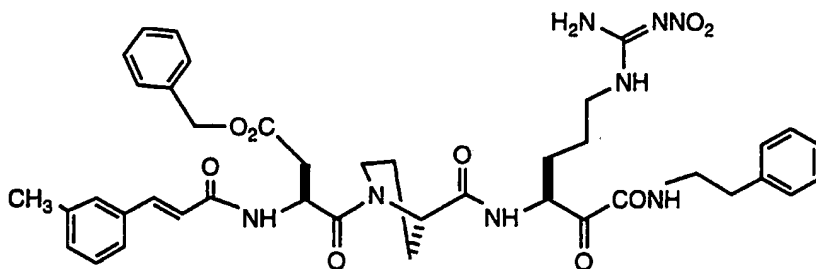
10

[53]

The compound of Example 6 (0.68 g, 1.23 mmole) was converted to the above-identified product using procedures as described in Example 9, using 3-methylcinnamic acid (in place of 4-methyl valeric acid). Flash chromatography (silica, 10% methanol/dichloromethane) afforded 0.582 g (59%) of the above-identified compound as a foam. $R_f = 0.34$ (10% methanol/dichloromethane).

15

20

Example 13Preparation of

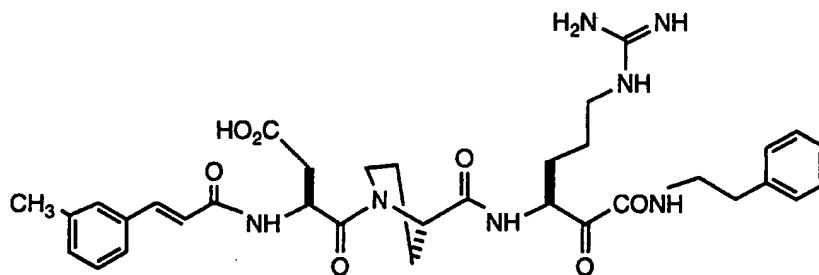
25

[54]

A 0.541 g portion of the compound of Example 12 (0.541 g, 0.675 mmole) was oxidized and worked up as described in Example 7. Flash chromatography (silica, 4:1:4 hexane/methanol/dichloromethane) afforded 0.462 g (85%) of the above-identified compound as a foam. R_f = 0.37 (10% methanol/dichloromethane).

Example 14

Preparation of



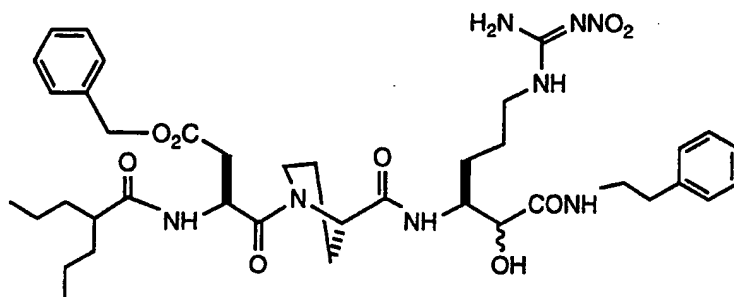
[55]

A 0.109 g portion of the compound of Example 13 (0.136 mmole) was transferred to an hydrofluoric acid reaction vessel. Anisole (0.1 mL) and a stir bar were added. The vessel was flushed with nitrogen and hydrofluoric acid and cooled to -20°C . Hydrofluoric acid (3.0 mL) was distilled into the reaction vessel with stirring. After 30 minutes, the vessel was warmed to 0°C and flushed with nitrogen. After 1 hour, the hydrofluoric acid was evaporated. The resulting material was extracted with water then 20% acetic acid/water. Both aqueous layers were washed with diethyl ether, frozen and lyophilized. The water extract afforded 10 mg of material and the acetic acid extract afforded 43 mg (58% total). The two fractions were combined and purified by preparative HPLC (reverse phase, 50 mL/minute, 10-60% acetonitrile/water with 0.1% trifluoroacetic acid, 40 minute program). Acetonitrile was removed under vacuum from the appropriate fraction. The remaining liquid was frozen and lyophilized to

74

afford the above-identified compound as a white fluffy powder. Mass spectral analysis showed the expected molecular ion at 661.3 (calc. 661.3).

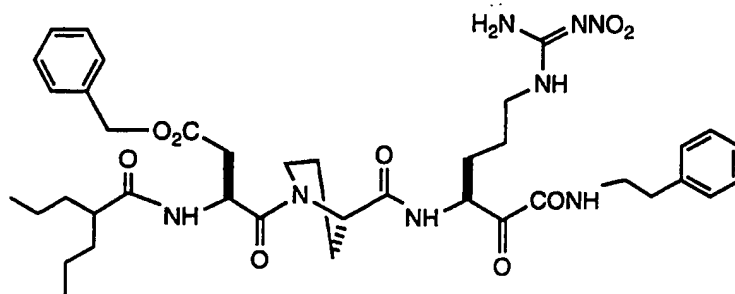
5 Example 15
Preparation of



10 [56]

A 0.637 g portion of the compound of Example 6 (1.15 mmole) was converted to the above-identified product using the methods described in Example 9 and using 2-propylpentanoic acid (in place of 4-methyl-valeric acid). Flash chromatography (silica, 10% methanol/dichloromethane) afforded 0.547 g (60%) of the above-identified compound as a foam. $R_f = 0.33$ (10% methanol/dichloromethane).

20 Example 16
Preparation of



[57]

25

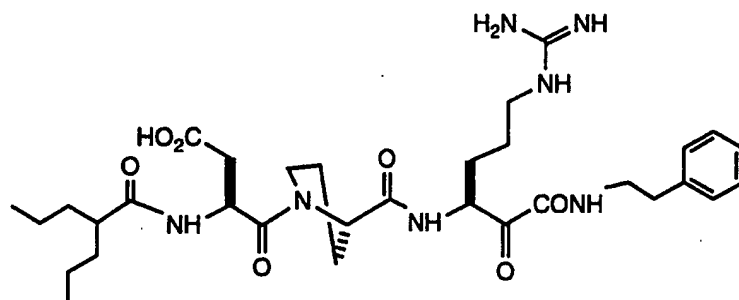
75

A 0.505 g portion of the compound of Example 15 (0.646 mmole) was oxidized and worked up as described in Example 7. Flash chromatography (silica, 4:1:4 hexane/methanol/dichloromethane) afforded 0.467 g (95%) of the above-identified compound as a foam. $R_f = 0.38$ (10% methanol/dichloromethane).

Example 17

Preparation of

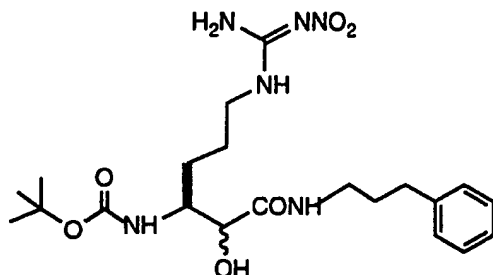
10



[4]

A 0.105 g portion of the compound of Example 16 (0.134 mmole) was deprotected using hydrofluoric acid as described in Example 15 to afford 30 mg (43%) of material. This material was purified by preparative HPLC (reverse phase, 50 mL/minute, 10-50% acetonitrile/water with 0.1% trifluoroacetic acid, 40 minute program). Acetonitrile was removed under vacuum from the appropriate fraction. The remaining liquid was frozen and lyophilized to afford the above-identified compound as a white fluffy powder. Mass spectral analysis showed the desired ion at 643.3 (calc. 643.4).

25

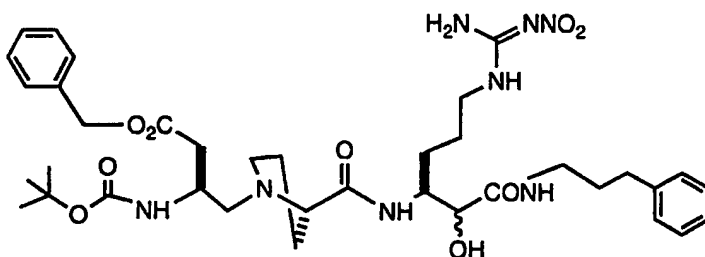
Example 18Preparation of

5

[58]

A 0.89 g portion of the compound of Example 3 (2.45 mmole) was converted to the amide as described in Example 5, using 3-phenylpropylamine (in place of 2-phenylethylamine). Flash chromatography (silica, 4:1:4 hexane/methanol/dichloromethane) afforded 1.03 g (90%) of the above-identified compound as a foam.

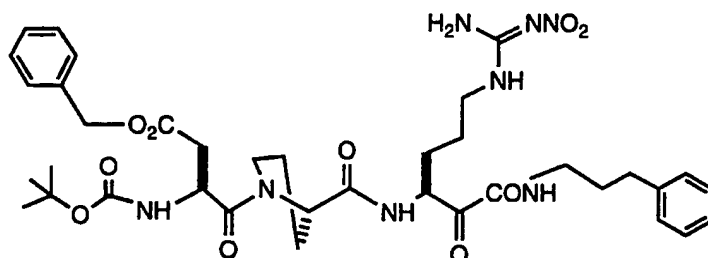
15 Example 19
Preparation of



[59]

20 A 0.5 g portion of the compound of Example 18 (1.07 mmole) was converted to the above product as described in Example 6. Flash chromatography (silica, 10% methanol/dichloromethane) afforded 0.735 g (89%) of the above-identified compound as a foam. $R_f = 0.27$ (10% methanol/dichloromethane).

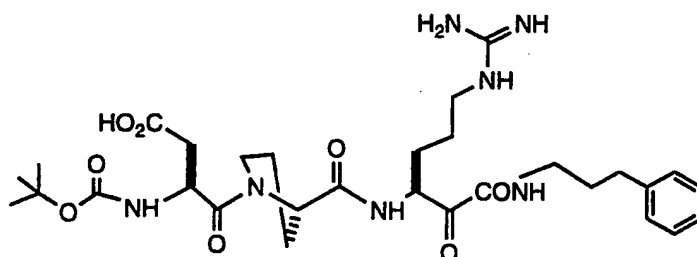
25

Example 20Preparation of

5

[60]

A 0.70 g portion of the compound of Example 19 (0.909 mmole) was oxidized and worked up as described in Example 7. Flash chromatography (silica, 4:1:4 hexane/methanol/dichloromethane) afforded 0.612 g (87%) of the above-identified compound as a foam. $R_f = 0.41$ (10% methanol/dichloromethane).

Example 2115 Preparation of

[5]

20 A 0.512 g portion of the compound of Example 20 (0.667 mmole) was hydrogenated and worked up as described in Example 8. The resulting foam was purified by preparative HPLC (reverse phase, 50 mL/minute, 20-60% acetonitrile/water with 0.1% trifluoroacetic acid, 40 minute program) The appropriate fractions were combined, the acetonitrile was removed under vacuum. The remaining liquid was frozen and lyophilized to afford

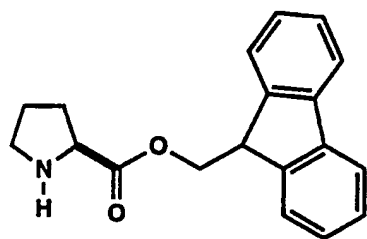
0.177 g (42%) of the above-identified compound as a white fluffy powder. Mass spectral analysis showed the expected molecular ion at 631.3 (calc. 631.3).

5 Example 22

Preparation of

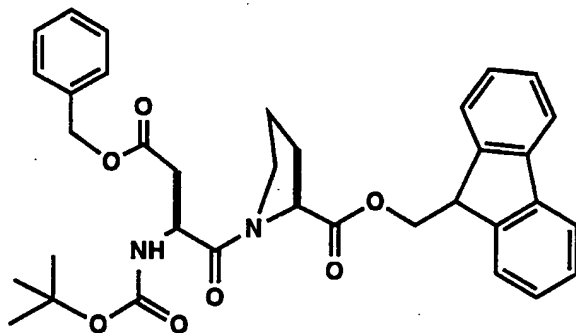
L-proline-9-fluorenemethyl

ester p-toluenesulfonic acid salt



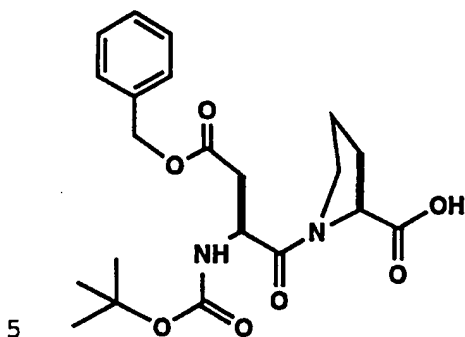
[61]

A solution of L-proline (15.99 g, 139.0 mmole), 9-fluorenemethanol (30.0 g, 152.9 mmole), and p-toluene-sulfonic acid in 600 mL of toluene was refluxed and water was removed with a Dean-Stark trap. After 26 hours, the reaction was concentrated to give 64 g (99% crude yield) of the above-identified compound as an oil which was used directly in the next step.

Example 23Preparation of α -N-(t-butoxycarbonyl)-L-aspartyl-(β -benzyl ester)-L-proline-9-5 fluorenemethyl ester

[62]

- 10 A solution of L-proline-9-fluorenemethyl ester p-toluenesulfonic acid salt (the product of Example 22) (15.44 g, 33.2 mmole), α -N-(t-butoxycarbonyl)-L-aspartic acid-(β -benzyl ester) (9.35 g, 41.9 mmole), benzotriazol-1-yloxy-tris-(dimethylamino)-phosponium-hexafluoro-
- 15 phosphate (18.6 g, 42.0 mmole) in 100 mL dimethylformamide was allowed to stir in an ice-bath. This solution was treated with 1-HOBt hydrate (0.45 g, 3.34 mmole), diisopropylethylamine (19.0 mL, 198 mmole) and the reaction allowed to stir at about 0 to 5°C for 1.5
- 20 hours. After this time the reaction mix was poured into 600 mL of ethyl acetate and extracted successively with saturated aqueous citric acid, water, saturated sodium bicarbonate, and finally brine. The organic phase was dried with anhydrous magnesium sulfate and concentrated
- 25 under vacuum to give 18 g (91% crude yield) of an oil, which was used directly in the next step.

Example 24Preparation of α -N-(t-butoxycarbonyl)-L-aspartyl-(β -benzyl ester)-L-proline

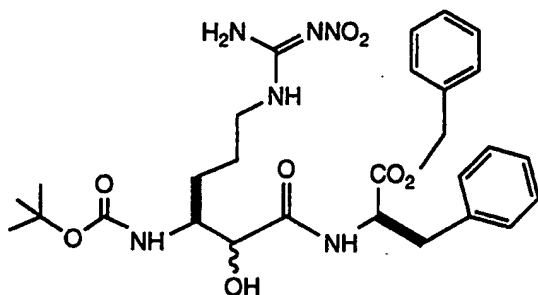
[63]

The crude oil from Example 23, α -N-(t-butoxycarbonyl)-L-aspartyl-(β -benzyl ester)-L-proline 9-fluorenemethyl ester (17.5 g, 29.2 mmole), was suspended in 250 mL triethylamine and allowed to reflux for 1 hour. This mixture was concentrated to an oil, dissolved in 600 mL of ethyl acetate. The ethyl acetate phase was washed once with a citric acid solution, once with brine, dried with anhydrous magnesium sulfate, and concentrated under vacuum to give an oil. This material was purified by column chromatography (silica gel, 10-20% tetrahydrofuran/dichloromethane) to give 7.5 g (yield about 38% overall) of the above-identified compound.

10

15

20

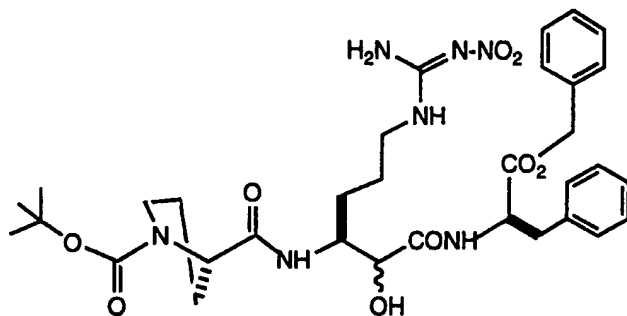
Example 25Preparation of

5

[64]

A solution of 400 mg of the compound of Example 4 (1.14 mmole) was dissolved with stirring into 2 mL of dimethylformamide. This solution was treated successively with D-phenylalanine benzyl ester p-toluenesulfonic acid salt (489 mg, 1.14 mmole), NMM (0.342 mL, 3.11 mmole) and BOP (5.15 mg, 1.16 mmole). After 2 hours, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no more material corresponding to the acid. The reaction mixture was poured into ethyl acetate (300 mL) and washed successively with 1N aqueous hydrochloric acid (75 mL), water (75 mL), saturated sodium bicarbonate (75 mL) and brine (75 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum. This afforded 600 mg (90%) of the above-identified compound as a white foam. $R_f = 0.70$ (two spots, 10% methanol/dichloromethane).

15
20

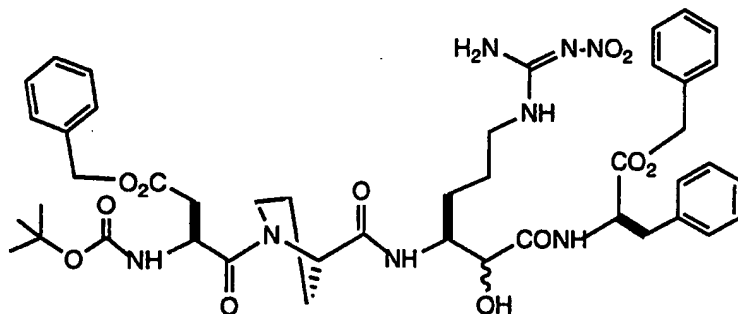
Example 26Preparation of

5

[65]

To a solution of 586 mg of the compound of Example 25 (1.00 mmole) in 17 mL dichloromethane, was added 17 mL of trifluoroacetic acid with stirring. After 30 minutes, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no starting material. The trifluoroacetate salt was isolated by concentrating the solution. The residue was dissolved in toluene and then concentrated to an oil, which had some trifluoroacetic acid. The oil was dissolved in 3 mL of dimethylformamide with stirring and this solution treated with (264 mg, 1.49 mmole) α -N-t-butoxycarbonyl-L-proline, 0.600 mL (6.7 mmole) NMM and 554 mg (1.23 mmole) BOP. After 1 hour, thin layer chromatographic analysis (10% methanol/dichloromethane) showed no more trifluoroacetate salt. The mixture was added to 300mL of ethyl acetate and washed successively with 75mL of 1N aqueous hydrochloric acid, 75 mL of water, 75 mL of saturated sodium bicarbonate and 75 mL of brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum. This afforded 700 mg of the above-identified compound as a white foam, R_f = 0.70 (two spots, 10% methanol/dichloromethane).

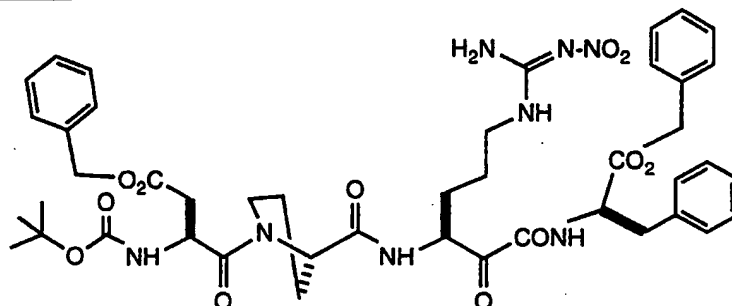
30

Example 27Preparation of

5

[66]

This compound was synthesized by the procedure described in Example 26, except that an equimolar amount of the compound of Example 26 and Boc-L-aspartic acid-
 10 (β -benzyl ester) was used, instead of α -N-t-butoxycarbonyl-L-proline. This gave 825 mg of the above-identified compound (R_f = 0.5, two spots, 10% methanol/dichloromethane).

15 Example 28Preparation of

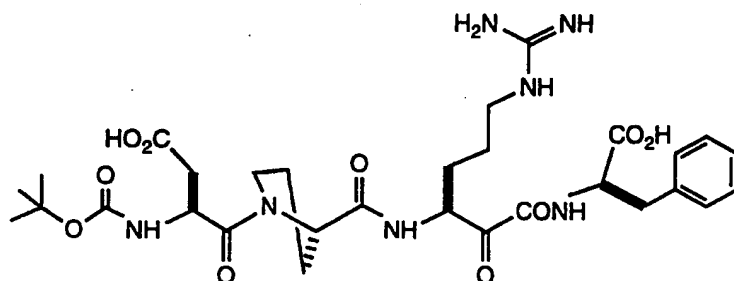
[67]

20 A 370 mg portion of the compound of Example 27 (0.42 mmole) was dissolved in 4 mL of dimethylsulfoxide with stirring. This solution was treated with 4 mL of toluene and 816 mg (4.26 mmole) of ethyl-3-(3-dimethylamino)-propylcarbodiimide hydrochloride followed

by 0.150 mL of dichloroacetic acid. After 1 hour, thin layer chromatographic analysis (10% methanol/dichloromethane) showed a single new spot and no starting material. The mixture was added to 500mL of ethyl acetate and washed with two 200 mL portions of water and 150 mL of brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated under vacuum. Flash chromatography (silica, 0 to 4% methanol/dichloromethane) afforded 200 mg (54 % yield) of the above-identified compound as a foam. $R_f = 0.55$ (10% methanol/dichloromethane).

Example 29

Preparation of



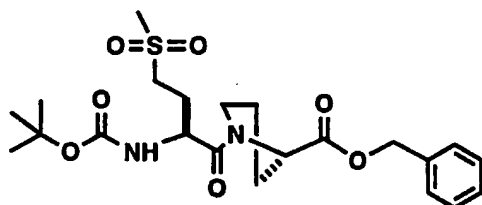
[68]

A 0.160 mg portion of the compound of Example 28 (0.180 mmole) was dissolved in 25 mL of methanol. This solution was added to a Parr vessel containing 150 mg 10% Pd/C, followed by 0.20 mL (0.20 mmole) 1N aqueous hydrochloric acid and 0.2 mL of glacial acetic acid. The mixture was shaken under a 10 psig hydrogen atmosphere for 1.5 hours, after which HPLC (reverse phase, 1 mL/minute, 5-95% acetonitrile/water with 0.1% trifluoroacetic acid, 20 minute program, retention time = 14.5 minute) showed complete reaction. The mixture was filtered, rinsed with 10 mL of methanol and concentrated under vacuum. The resulting foam was purified by preparative HPLC (reverse phase, 50 mL/minute, 10-60%

acetonitrile/water with 0.1% trifluoroacetic acid, 40 minute program). The appropriate fractions were combined and the acetonitrile was removed under vacuum. The remaining liquid was frozen and lyophilized to afford 100 mg of the above-identified compound as a white fluffy powder. Mass spectral analysis showed the expected molecular ion at 661.3 (calc. 661.3).

Example 30

10 Preparation of

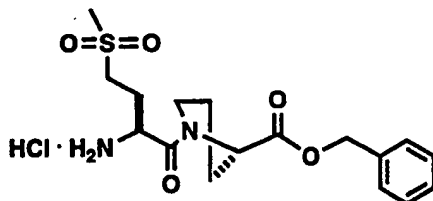


[69]

15 To a solution of t-butoxycarbonylmethioninesulfone acid (14.0 g, 50.0 mmole) in dichloromethane (150 mL) at 0°C was added HOBt (10.1 g, 75 mmole) followed by dicyclohexylcarbodiimide (11.33 g, 55.0 mmole). The mixture was stirred for 10 minutes, and then proline
20 benzyl ester hydrochloride salt (50.0 mmole, 12.0 g) was added followed by NMM (100 mmole, 10.9 mL). The resulting mixture was stirred in an ice bath and allowed to come to room temperature over 12 hours. The mixture was then filtered to remove dicyclohexylurea and ethyl
25 acetate (300 mL) is added. The organic phase was then added to a separatory funnel and washed with saturated aqueous sodium bicarbonate, brine and then 1 M aqueous HCl. The organic phase was dried over magnesium sulfate and then filtered. The organic phase was then reduced
30 on a rotary evaporator in vacuo and then on a high vacuum line to remove traces of solvent to provide 23.5 g of a white solid (100%). Rf=0.34 (silica gel, trichloromethane/methanol (95:5)).

Example 31

Preparation of



[70]

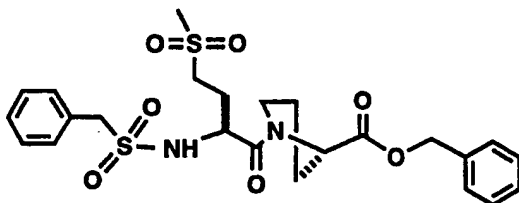
To a solution of t-butoxycarbonylmethioninesulfone-proline benzylester (23.5 g, 50 mmole) in dry dioxane (300 mL) was added 100 mL of a 4 M HCl dioxane solution.

The mixture was then stirred at room temperature for 1 hour until the starting material disappeared as shown by thin layer chromatography analysis (10% trichloromethane:methanol). The diethyl ether was added to the mixture to precipitate the white hydrochloride salt.

The mixture was filtered on a Buchner funnel and then dried under high vacuum to provide 20.16 g (100%) of a white solid.

Example 32

Preparation of



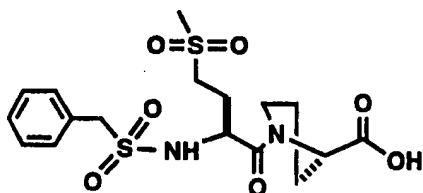
[71]

To a solution of methioninesulfoneproline benzylester hydrochloride (20.0 mmole, 8.08 g) in dry acetonitrile (100 mL) cooled to 0°C was added α -toluenesulfonylchloride (20.0 mmole, 3.8 g) all at once followed by pyridine (50.0 mmole, 4.2 mL). The mixture was then stirred in the ice bath for 12 hours eventually

warming to room temperature. Work-up consisted of reducing the volume in vacuo and diluting with ethyl acetate (300 mL). The organic phase was then washed with saturated aqueous sodium bicarbonate, brine and 1 M aqueous HCl (100 mL). The organic phase was dried over magnesium sulfate, filtered and evaporated in vacuo to provide 8.8 g (100%) of a foamy golden solid. $R_f=0.31$ (silica gel, trichloromethane:methanol (95:5)). The solid was filtered through a plug of silicon dioxide (50 g) using ethylacetate before hydrogenation to eliminate possible sulfur related impurities.

Example 33
Preparation of

15

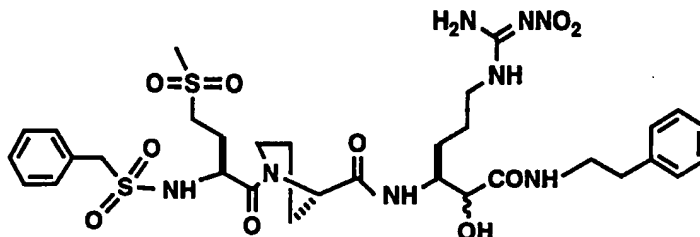


[72]

To a solution of α -toluenesulfonylmethionine-sulfoneproline benzylester (8.8 g, 20 mmole) in methanol (300 mL) was added 1.0 g of 10% Pd/C. The mixture was then hydrogenated at 1 atmosphere of hydrogen gas and room temperature. The mixture was stirred for 12 hours. The mixture was then filtered and the organic phase reduced in vacuo to provide 8.0 g (100%) of a white foamy solid.

Example 34Preparation of

5 [73]

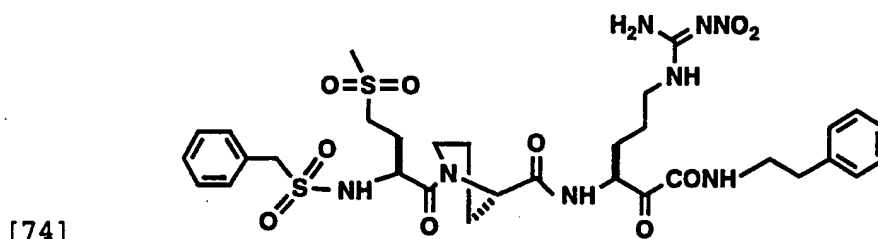


A 600 mg (1.1 mmole) portion of the product of Example 5 was taken up in trifluoroacetic acid at 0°C, and stirred for two hours. This solution was diluted with toluene (100 mL) and reduced in vacuo. This residue was dissolved in dimethylformamide (6 mL) and 361 mg (1.1 mmole) of α -toluenesulfonylglycineproline were added followed by 538 mg (1.22 mmole) of BOP and 1117 mg (11.0 mmole, 1.21 mL) of NMM and the solution was allowed to stir overnight.

This solution was diluted in 50 mL 1 M HCl and extracted three times with ethyl acetate. The organics were combined and washed with water (three times), saturated sodium bicarbonate and brine. The solution was dried over magnesium sulfate and concentrated in vacuo to give 405 mg of the above compound as an orange/yellow foam. Thin layer chromatography showed no more starting material.

Example 35

Preparation of



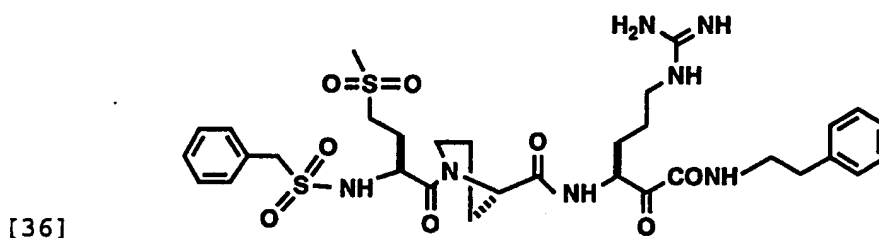
5

A 400 mg (0.605 mmole) portion of the product of the previous example, was taken up in 10 mL of 1:1 toluene:DMSO with EDC. To this solution was added 312 mg (0.2 mL, 2.42 mmole, 4.0 eq) of dichloroacetic acid. This solution was stirred for one hour and ten minutes, diluted with 50 mL water and extracted twice with ethyl acetate (100 mL). The organics were combined, washed with brine, dried over magnesium sulfate, and concentrated in vacuo. This solution was purified on a (4:1:4 hexanes:methanol:dichlormethane) silica column to give 150 mg of a (clean) white solid.

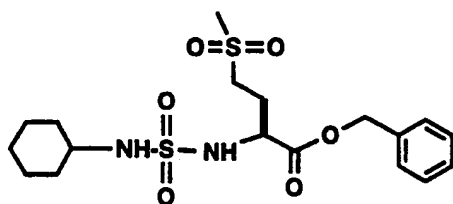
Example 36

Preparation of

20

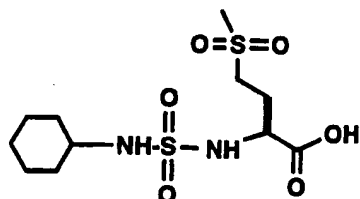


The product of the previous example was subjected to hydrogen fluoride as previously described and purified by HPLC to give the above compound which had an actual mass spectra peak (613.2) that correlated well with the expected value of 613.3..

Example 37Preparation of

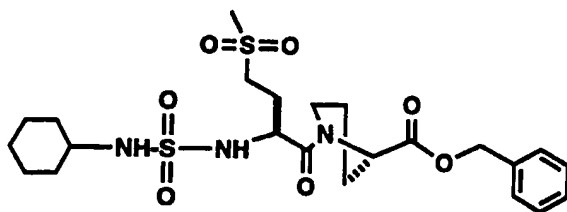
5 [75]

To cyclohexylamine sulfamic acid sodium salt (Aldrich, 2.01 g, 10.0 mmole) was added 6 mL phosphorousoxychloride. The white suspension was then heated
10 to 100°C for 4 hours. The mixture was then cooled to room temperature and the phosphorousoxychloride was stripped off in vacuo to provide a white solid. This solid was then suspended in dry acetonitrile (35 mL) and then cooled to 0°C. To this mixture was added the
15 methionine sulfone benzyl ester hydrochloride salt of Example 31 (3.07 g, 10.0 mmole) followed by pyridine (2.6 mL, 30.0 mmole). The mixture was allowed to warm to room temperature in the ice bath over 10 hours. The acetonitrile was stripped off in vacuo and then diluted
20 with ethyl acetate. The organic phase was washed with saturated aqueous sodium bicarbonate, brine and 1 M aqueous HCl. The organic phase was dried over magnesium sulfate, filtered and reduced in vacuo to provide 4.8 g of a crude yellow solid. The solid was washed with
25 ethyl ether and then filtered to provide 3.8 g (88%) of an off white solid.

Example 38Preparation of

5 [76]

The product of the previous example was dissolved in 100 mL of a 1:1 mixture of tetrahydrofuran/methanol and 0.5 g of 10% Pd/C was added. The mixture was hydrogenated at 1-atmosphere of hydrogen for 4 hours at room temperature. The mixture was then filtered and reduced in vacuo to provide 3.2 g of a white solid.

Example 3915 Preparation of

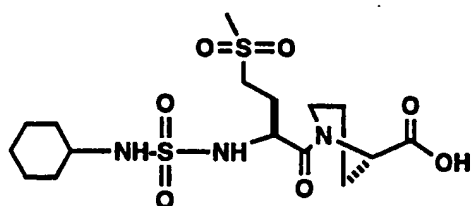
[77]

20 To a solution of the cyclohexylsulfonylureamethionine-sulfone acid product of Example 33 (2.75 g, 8.0 mmole) in dry dimethylformamide (10 mL) was added EDC (8.0 mmole, 1.53 g) and HOBT (12 mmole, 1.62 g) all at once. This mixture was stirred at 0°C for 10 minutes, then proline benzylester hydrochloride (8 mmole, 1.93 g) was added followed by NMM (24 mmole, 2.6 mL). The reaction was allowed to come to room temperature in an ice bath over 10 hours. The reaction mixture was then diluted with ethyl acetate and washed with saturated

aqueous sodium bicarbonate, brine and 1 M aqueous HCl. The organic phase was dried over magnesium sulfate, filtered and reduced in vacuo to provide 3.25 g (75%) of a viscous foamy solid. $R_f=0.19$ (silica gel, trichloromethane:methanol (95:5)).

Example 40

Preparation of

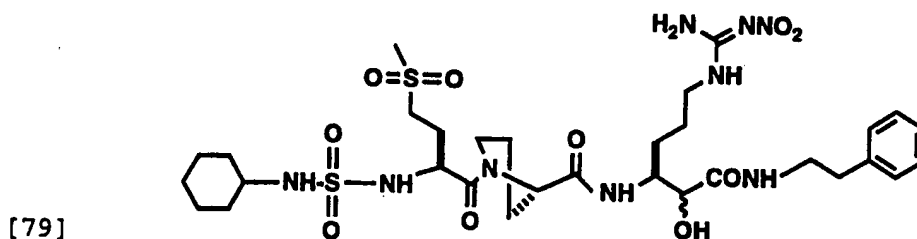


[78]

To a solution of the benzyl ester product of Example 36 (3.2 g, 6.0 mmole) in methanol (60 mL) was added 15 mL of a 2.0 M lithium hydroxide solution at room temperature. The clear solution was stirred for 1 hour and then the methanol was stripped off in vacuo. The aqueous solution was then washed with ethyl ether (2x100 mL) and then the aqueous solution was neutralized to pH 1 with 1 M aqueous HCl and extracted twice with 100 mL ethyl acetate. The organic phase was then washed with brine, dried over magnesium sulfate, filtered and reduced in vacuo to provide 2.3 g (90%) of a white fluffy solid. $R_f=0.13$ (silica gel, trichloromethane:methanol (70:30)).

Example 41

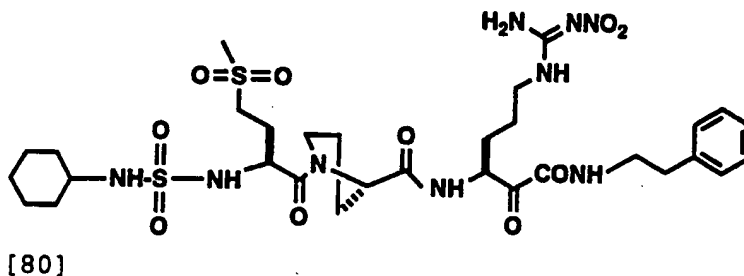
Preparation of



The product of the previous example and the product of Example 5 were coupled as described in various examples herein.

10 Example 42

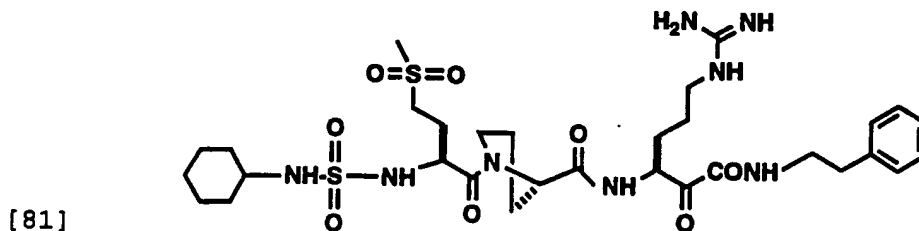
Preparation of



The product of the previous example was oxidized and worked up as described in Example 35.

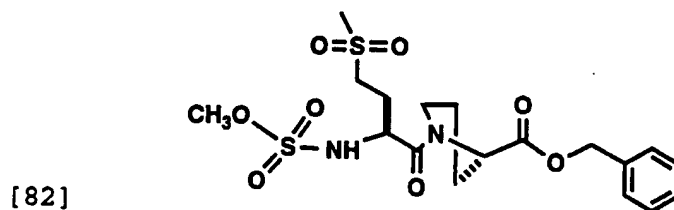
Example 43

20 Preparation of



The product of the previous example was subjected to hydrogen fluoride as previously described and purified by HPLC to give the above compound.

5 Example 44
Preparation of

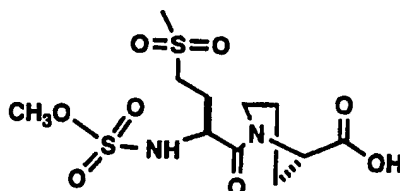


- 10 To methyl sulfate sodium salt (Aldrich, 1.34 g, 10.0 mmole) is added 10 mL phosphorousoxychloride and the mixture heated to 100°C for 3 hours. The reaction is cooled to room temperature and the phosphorousoxychloride removed in vacuo to leave a white residue. The
- 15 residue is then mixed with dry acetonitrile (25 mL) and cooled to 0°C in an ice bath. Then the methionine-sulfoneproline benzylester hydrochloride salt (4.04 g, 10 mmole) is added all at once followed by pyridine (2.6 mL, 30 mmole). The reaction is allowed to warm to room
- 20 temperature in the ice bath over 10 hours. The acetonitrile is removed in vacuo and the residue diluted with ethyl acetate. The organic phase is washed with saturated aqueous sodium bicarbonate, brine and 1 M aqueous HCl. The organic phase is dried over magnesium sulfate,
- 25 filtered and the solvent removed in vacuo to provide the coupled product in a yield of 4.6 g (100%).

95

Example 45Preparation of

[83]



5

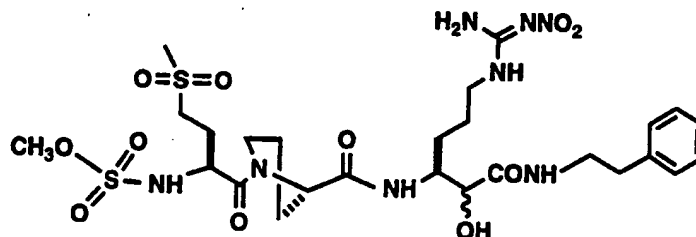
The product of the previous example is dissolved in 150 mL of methanol and 0.5 g of 10% Pd/C is added. The mixture is then hydrogenated at atmospheric pressure for 4 hours. The mixture is filtered and the solvent removed in vacuo to provide the corresponding acid in a yield of 3.6 g (100%).

10

Example 46Preparation of

15

[84]

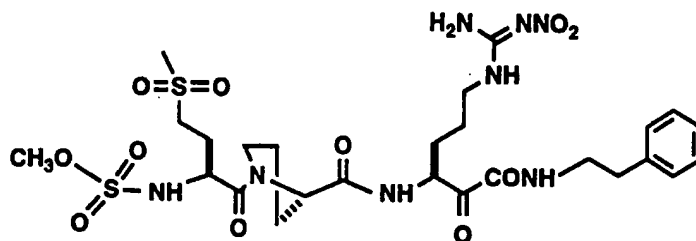


The product of Example 5 and the product of the previous example are coupled as described in various examples herein.

20

Example 47Preparation of

[85]

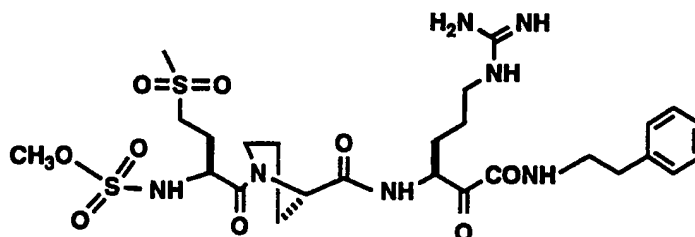


5

The product of the previous example was oxidized and worked up as described in Example 35.

Example 4810 Preparation of

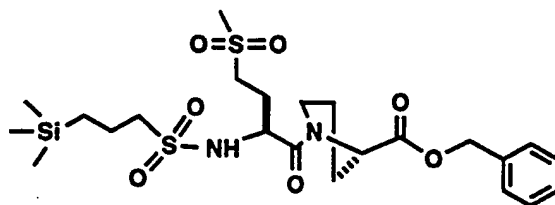
[86]



15 The product of the previous example is subjected to hydrogen fluoride as previously described and purified by HPLC to give the above compound.

Example 4920 Preparation of

[87]



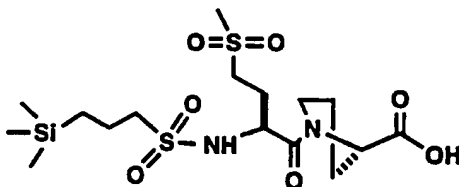
To 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (available from Aldrich or Hüls America) (5.0 g,

23.0 mmole) was added 10 mL phosphorousoxychloride . The mixture was then heated to 100°C for 3 hours and then cooled to room temperature. The phosphorousoxychloride was then removed in vacuo and the residue partitioned between ethylacetate and ice. After the ice melted the ethyl acetate phase was separated and washed three times with saturated aqueous sodium bicarbonate until pH 8 by litmus paper was observed. The ethyl acetate was then washed with brine and dried over magnesium sulfate, filtered and the solvent removed in vacuo to provide 4.04 g (82%) of a yellowish oil. Rf=0.52 (silica gel; hexanes:ethyl acetate (90:10)).

To a solution of the above 3-(trimethylsilyl)-1-propanesulfonyl chloride (2.14 g, 10.0 mmole) in dry acetonitrile (20 mL) cooled to 0°C in an ice bath was added the methioninesulfoneproline benzylester hydrochloride (4.04 g, 10.0 mmole) followed by pyridine (2.55 mL, 30.0 mmole). The mixture was allowed to warm to room temperature in the ice bath over the course of 10 hours. The acetonitrile was then stripped off in vacuo and the residue diluted with ethyl acetate. The organic phase was washed with saturated aqueous Sodium bicarbonate, brine and 1 M aqueous HCl. The organic phase was then dried over magnesium sulfate, filtered and the solvent removed in vacuo to provide 4.85g (89%) of a viscous oil. Rf=0.23 (silica gel, trichloromethane:methanol (95:5)).

Example 50

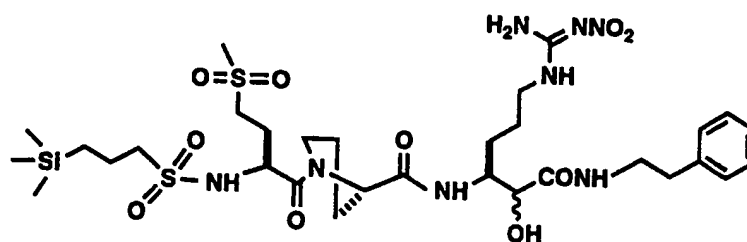
Preparation of



To a solution of 3-(trimethylsilyl)-1-propane-sulfonyl methionine sulfone proline benzylester (4.8 g, 8.8 mmole) in methanol (150 mL) was added 25 mL of a 2.0 M lithium hydroxide solution. The mixture was stirred at room temperature for 1 hour and then the methanol was removed in vacuo. The aqueous phase was washed twice with 100 mL ethyl ether and then neutralized to pH 1 with 1 M aqueous HCl. The aqueous phase was extracted, filtered, and the solvent removed in vacuo to provide 3.24 g (81%) of the corresponding acid. R_f=0.25 (silica gel, trichloromethane:methanol (70:30)).

Example 51

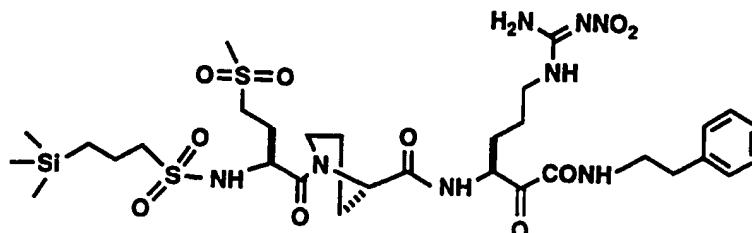
Preparation of



The product of Example 5 and the product of the previous example were coupled as described in various examples herein.

Example 52

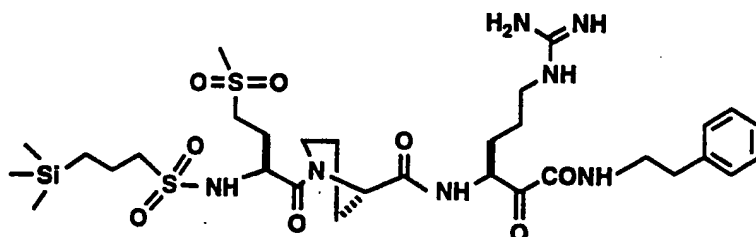
Preparation of



The product of the previous example was oxidized and worked up using the procedure of Example 74.

Example 53Preparation of

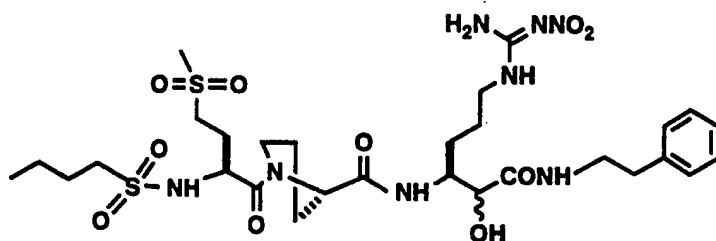
5 [91]



To a solution of the compound of the previous example (8.8 g, 20 mmole) in methanol (300 mL) was added 1.0 g of 10% Pd/C. The mixture was then hydrogenated at 1 atmosphere and room temperature. The mixture was stirred for 12 hours. The mixture was then filtered and the organic phase reduced in vacuo to provide 8.0 g (100%) of a white foamy solid.

15 Example 54Preparation of

[92]



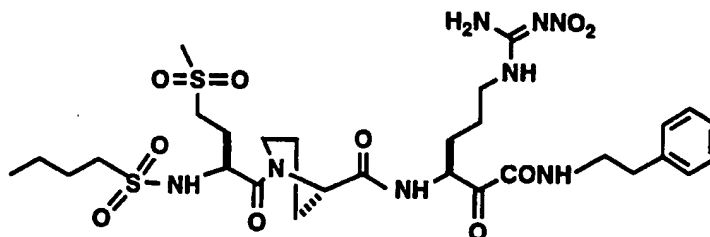
20 A 6.0 g (12.4 mmole) portion of the product of methioninesulfoneproline benzylester trifluoroacetic acid salt of the previous example was reacted with 2.07 mL (16 mmole) of n-butesulfonylchloride and 5.0 mL (36 mmole) of triethylamine in dichloromethane from 0°C to room temperature. The reaction mixture was poured into saturated aqueous bicarbonate and extracted with ethyl acetate (2 x 100 mL). The organic phase was washed with brine and 1 M aqueous HCl. The organic phase was sepa-

rated and dried over magnesium sulfate, filtered and reduced in vacuo to give 5.73 g of viscous oil. The oil was mixed with 2 M potassium hydroxide (20 mL) and 100 mL methanol at room temperature for two hours. The
5 methanol was reduced in vacuo and the aqueous solution was then washed with ether (2 x 50 mL) and then neutralized with 1 M HCl to a pH of 1. The aqueous solution was then extracted with ethyl acetate (2 x 100 mL) and dried over magnesium sulfate, filtered and reduced in
10 vacuo to give 2.85 g of the above acid as a viscous foamy solid. The overall yield was 60.5%.

A 500 mg (1.1 mmole) portion of the product of Example 5 was taken up in trifluoroacetic acid at 0°C and stirred for two hours. This mixture was diluted
15 with toluene (100 mL) and concentrated in vacuo two times. The residue was taken up in dimethylformamide. To this solution were added 440 mg (1.1 mmole, 1.0 equivalents) of the above acid of the previous paragraph, 1.21 mL (1117 mg, 11.0 mmole, 10.0 equivalents)
20 of NMM and 538 mg (1.22 mmole, 1.1 equivalents) of BOP. This solution was stirred overnight. The solution was diluted in 120 mL of 1 M HCl and extracted three times with 50 mL of ethyl acetate. The organic phases were combined, washed with 1 M HCl, water (three times),
25 saturated sodium bicarbonate and brine and then dried over magnesium sulfate. It was concentrated in vacuo to give 380 mg of the above crude product.

Example 55Preparation of

[93]

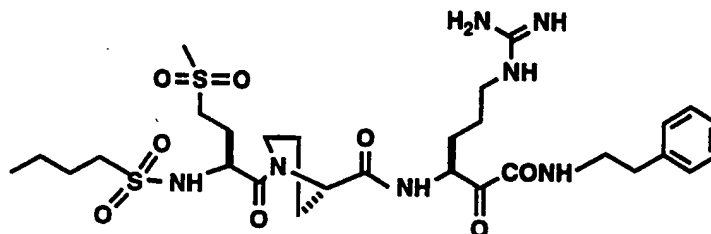


5

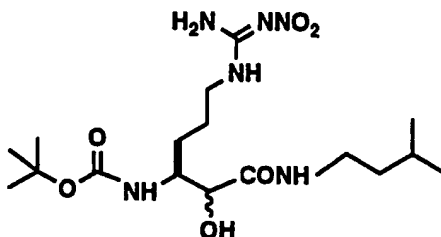
The product of the previous example was taken up in 10 mL of 1:1 PheMe:DMSO and 994 mg (5.19 mmole, 10.0 equivalents) of EDC and 0.17 mL (267 mg, 2.07 mmole, 4.0 equivalents) dichloroacetic acid were added. The reaction mixture was allowed to stir for about one hour and ten minutes and then diluted with water (about 50 mL). It was extracted twice with ethyl acetate. The organic phases were combined, washed with brine, dried over magnesium sulfate and concentrated in vacuo. The concentrate was purified on a (4:1:4 hexanes:methanol:dichloromethane) silica column, to give 80 mg of the above compound.

20 Example 56
Production of

[94]



25 The product of the previous example was cleaved with hydrogen fluoride and purified by HPLC to give the above product with an observed mass spectra peak of 685.3 that agreed exactly with the expected value.

Example 57Preparation of

[95]

5

A 10 g (27.5 mmole, 1.0 equivalent) portion of the product of Example 5 was taken up in methanol and 34.4 mL (34.4 mmole, 1.25 equivalents) of lithium hydroxide were added. This solution was allowed to stir overnight. The solution was concentrated to 90 mL, in vacuo diluted with 500 mL of water, and extracted three times with ethyl acetate. The aqueous phase was then concentrated to about 400 mL, and gravity filtered through Dowex 50 resin (150 mL bed in a sintered glass funnel)

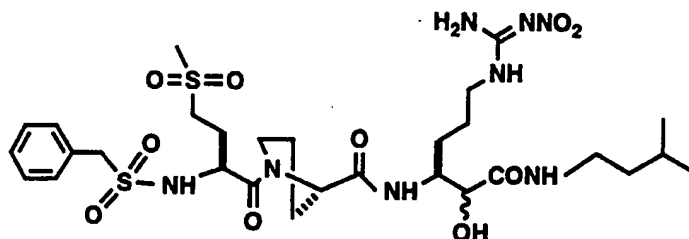
The resin was washed with 800 mL of water and 500 mL of 50:50 methanol/water until no more UV active resin material was observed. The material was concentrated in vacuo, and reconstituted twice with acetonitrile. Then 2.0 g (5.71 mmole) of this material was taken up in 28.5 mL of dimethylformamide.

To this solution were added 0.663 mL (500 mg, 5.71 mmole, 1.0 equivalents) of isoamylamine, 1.88 mL (1733 mg, 17.13 mmole, 3.0 equivalents) of NMM, and 2778 mg (6.28 mmole, 1.1 equivalents) of BOP. This solution was allowed to stir over 48 hours.

This solution was diluted in 1 M HCl and extracted three times with ethyl acetate. The organics were recombined, washed with 1 M HCl (once), water (three times), saturated sodium bicarbonate, and brine. The solution was then dried over magnesium sulfate and concentrated in vacuo to 1.5 g of the above compound.

Example 58Production of

5 [96]

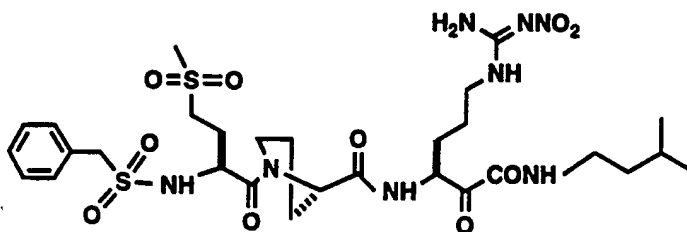


The product of the previous example was reacted with the product of Example 33 using the procedure of Example 59 to give the above compound.

10

Example 59Production of

[97]

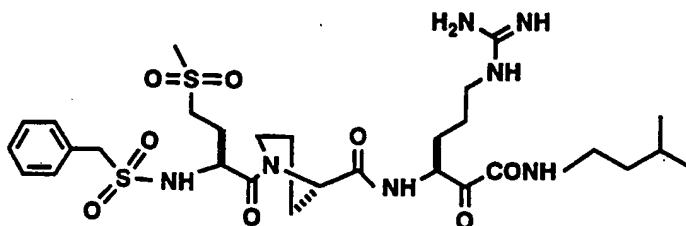


15

The product of the previous example was oxidized and worked up to give the above compound.

Example 6020 Production of

[98]



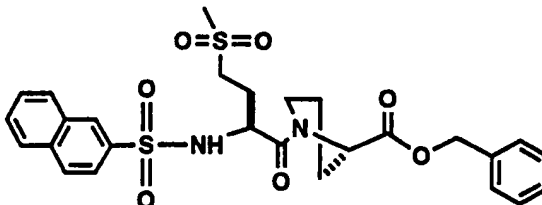
104

The product of the previous example was cleaved with hydrogen fluoride to give the above compound.

Example 61

5 Preparation of

[99]

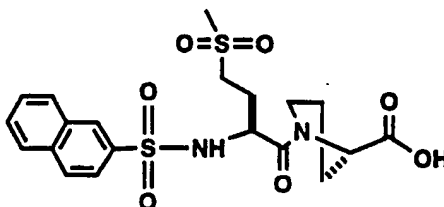


A 3 g (6.878 mmole) portion of the product of
10 Example 31 was added to 69 mL of acetonitrile. To this
mixture was added 2.339 g (10.317 mmole, 1.5 eq) of 2-
naphthylsulfonylchloride and 4.201 g (4.115 mL, 34.39
mmole, 5 eq) of pyridine and stirred for 10 hours. This
mixture was concentrated in vacuo and diluted with ethyl
15 acetate (500 mL) followed by washing with 1 M HCl,
water, aqueous sodium bicarbonate, and brine. The
organic phase was dried over magnesium sulfate and con-
centrated in vacuo. Thin layer chromatography (10%
methanol/dichloromethane) showed some 2-naphthylsul-
20 fonylchloride. The mixture was then filtered (silica,
dichloromethane (100 mL) then 10% methanol/
dichloromethane (200 mL) to give 3.96 g of the above
compound

25 Example 62

Preparation of

[100]



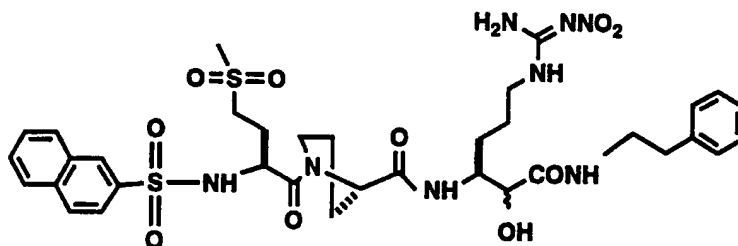
105

A 3.96 g (7.088 mmole) portion of the above product was dissolved in 250 mL of methanol with a trace of tetrahydrofuran. To this solution 2 g of 10% Pd/C was added under nitrogen and stirred under hydrogen at one atmosphere of pressure. Thin layer chromatography (10% methanol/dichloromethane) showed no starting material. This solution was then filtered through a nylon filter and concentrated in vacuo to give the above compound. Yield was 3.2 g (96%).

Example 63

Preparation of

[101]

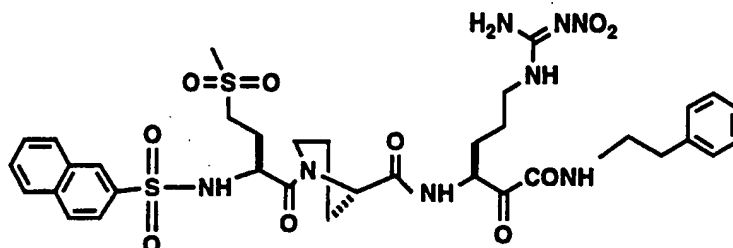


A 0.603 g (1.288 mmole) portion of the product of the previous example and a 0.5 g (1.288 mmole) portion of nitroarginine- α -hydroxy-2-phenylethylamide hydrochloride salt were dissolved in 13 mL of dimethylformamide with stirring. To this solution was added 0.651 g (0.708 mL, 6.44 mmole) of NMM and 566 mg BOP and the reaction stirred 10 hours. This solution was extracted with ethyl acetate (600 mL), 200 mL of water, 200 mL of 1 M HCl, 200 mL of water, 200 mL of sodium bicarbonate and 200 mL of brine; it was dried over magnesium sulfate and concentrated in vacuo. Thin layer chromatography (10% methanol/dichloromethane) showed no starting material. The above compound was obtained in a yield of 0.71 g (71%).

106

Example 64Preparation of

[102]

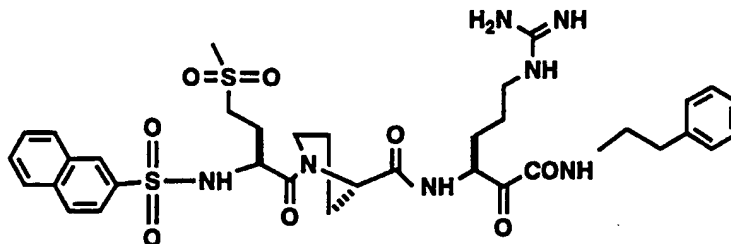


5

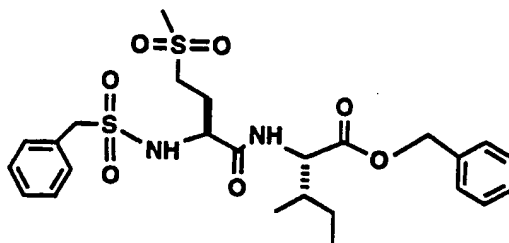
The 0.71 g of the product of the previous example was dissolved in 18 mL of 1:1 solution of toluene/DMSO with stirring. To this solution were added 1.695 g (8.84 mmole) of EDC and 0.456 g (0.292 mL, 3.536 mmole) of dichloroacetic acid. After 1.5 hours, thin layer chromatography (10% methanol/dichloromethane) showed no starting material. This solution was extracted with 500 mL of ethyl acetate, 200 mL of water, 200 mL of sodium bicarbonate and 200 mL of brine; it was dried over magnesium sulfate and concentrated in vacuo. Purification on a silica column using a 4:1:4 hexanes:methanol:dichloromethane as eluent gave 0.688 g (0.884 mmole) of the above compound.

20 Example 65Preparation of

[37]



25 The product of the previous example was subjected to hydrogen fluoride as previously described and purified by HPLC to give the above compound.

Example 66Preparation of

5 [103]

A 5 g (20.071 mmole) portion of t-butoxycarbonyl-methioninesulfone acid was dissolved in 80 mL of dimethylformamide with stirring. To this solution were
10 added 7.898 g (20.071 mmole) of isoleucine benzylester paratoluenesulfonic acid salt, 6.091 g (6.621 mL, 60.213 mmole, 3 equivalents) of NMM and 8.877 g (20.071 mmole, 1 equivalent) of BOP. This solution was extracted with 600 mL of ethyl acetate, 200 mL of water, 200 mL of HCl,
15 200 mL of water, 200 mL of aqueous sodium bicarbonate, and 200 mL of brine; it was dried over magnesium sulfate and concentrated in vacuo. Thin layer chromatography (10% methanol/dichloromethane showed no more starting material. To this solution was added 100 mL of 4M
20 HCl/dioxane. After approximately five hours thin layer chromatography showed no starting material. The solution was concentrated to give methioninesulfone-isoleucine benzylester hydrochloride in a yield of 8.9 g (98%).

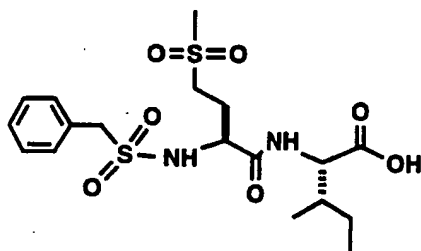
25 A 2.37 g (5.641 mmole) portion of the above salt and a 1.613 g (8.462 mmole, 1.5 equivalent) portion of α -toluenesulfonylchloride were mixed with stirring in acetonitrile. To this solution was added 3.446 g (28.205 mmole, 5 equivalent) portion of pyridine and the
30 reaction stirred for 10 hours. This solution was concentrated, in vacuo and extracted with 600 mL of ethyl acetate, 100 mL of water, 100 mL of HCl, 100 mL of

108

- water, 100 mL of aqueous sodium bicarbonate and 100 mL of brine. This solution was then dried, filtered and concentrated. Thin layer chromatography (10% methanol/dichloromethane) showed a second spot. The solution was
- 5 filtered (silica, dichloromethane - 10% (100 mL) then methanol/dichloromethane (200 mL)). This gave compound 80 in a yield of 7.87 g (95%).

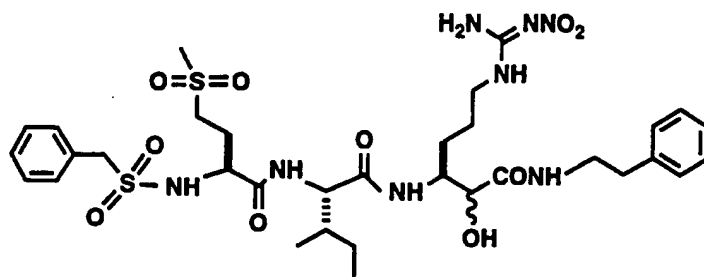
Example 67

10 Preparation of



[104]

- 15 The 2.87 g (5.333 mmole) of the product of the previous example was dissolved in 150 mL of methanol and 100 mL of tetrahydrofuran with stirring. The solution was purged with nitrogen, and 1.5 g of Pd/C was added and stirred under 1 atmosphere of hydrogen overnight.
- 20 The solution was filtered, and concentrated. This gave the above compound in a yield of 0.46 g (20%).

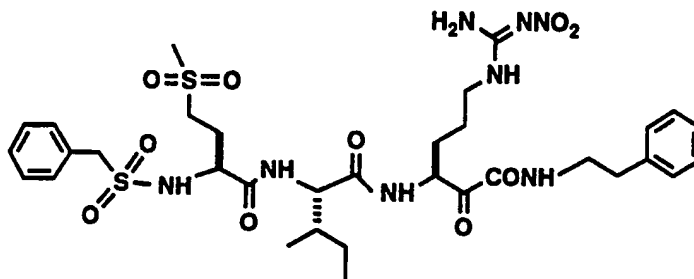
Example 68Preparation of

5 [105]

A 0.5 g (1.288 mmole) portion of the nitroarginine-
a hydroxy-2-phenylethyl hydrochloride salt of the
previous example and a 0.577 g (1.288 mmole) portion of
10 the product of the previous example were dissolved in 6
mL of dimethylformamide with stirring. To this solution
were added 0.651 g (6.44 mmole, 5 equivalents) of NMM
and 0.57 g (1.288 mmole, 1 equivalent) of BOP and the
reaction stirred for 10 hours. This solution was
15 extracted with 500 mL of ethyl acetate, 100 mL of water,
100 mL of HCl, 100 mL of water, 100 mL of aqueous sodium
bicarbonate, and 100 mL of brine; it was dried over mag-
nesium sulfate and concentrated. Thin layer chromatog-
raphy (10% methanol/dichloromethane) showed a minor con-
20 taminant. This gave the above compound in a yield of
0.541 g (54%).

Example 69

Preparation of



[106]

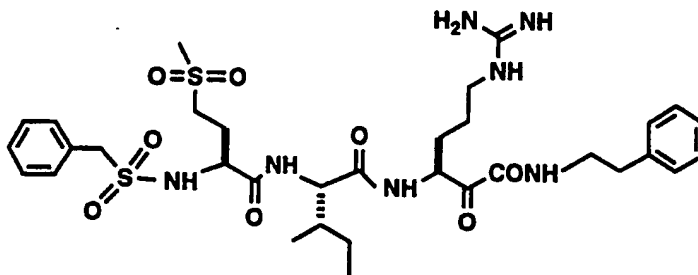
5

The 0.541g (0.691 mmole) of the product of the previous example was dissolved in 14 mL of 1:1 toluene/DMSO with stirring. To this solution were added 1.325 g (6.91 mmole, 10 equivalents) of EDC and 0.356 g (0.225 mL, 2.764 mmole, 4 equivalents) of dichloroacetic acid. After one hour thin layer chromatography (10% methanol/dichloromethane) showed no starting material.

The solution was extracted with 300 mL of ethyl acetate, 200 mL of water, 200 mL of water, 150 mL of aqueous sodium bicarbonate and 150 mL of brine; it was dried over magnesium sulfate and concentrated. This gave the above compound in a yield of 80 mg (15%).

Example 70

20 Preparation of



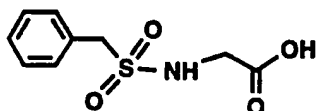
[107]

111

The product of the previous example was treated with hydrogen fluoride as in Example 14 and then purified using HPLC to give the above compound.

5 Example 71

Preparation of



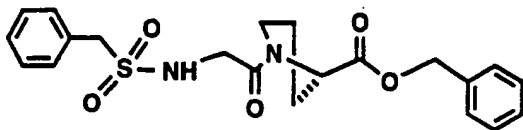
[108]

10

To a suspension of glycine ethyl ester hydrochloride (8.8g, 60.0 mmole) in dry hydrogen fluoride (150 mL) cooled to 0°C in an ice bath was added the α -toluenesulfonyl chloride (9.5 g, 50.0 mmole) followed by pyridine (12.1 mL, 150 mmole). The reaction was stirred in the ice bath over 10h allowing the reaction to warm to room temperature. The hydrogen fluoride was then removed in vacuo and the resulting residue diluted with ethylacetate. The organic solution was then washed with saturated aqueous sodium bicarbonate, brine then 1 M aqueous HCl. The organic phase was dried (MgSO₄), filtered and then reduced in vacuo to provide 6.67g reddish oil (52%). Rf=0.83 (3:2, Hexanes:Ethyl Acetate).

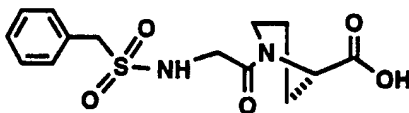
25 The above oil was diluted with MeOH (70 mL) and 30 mL of a 1.5 M LiOH aqueous solution was added. The mixture was stirred at 25°C for 1 hour. The MeOH was then reduced in vacuo to provide 3.14g (53%) of a tan solid. Rf=0.25 (70:30; CHCl₃:MeOH).

30

Example 72Preparation of

5 [109]

To a solution of the α -Toluenesulfonylglycine acid (1.5g, 6.5 mmole) in CH_2Cl_2 (25 mL) cooled to 0°C was added HOBt (1.3g, 9.8 mmole) followed by DCC (1.5g, 7.2 mmole). This mixture is stirred for 10 minutes at which time prolinebenzyl hydrochloride (1.74g, 7.2 mmole) was added followed by NMM (1.1 mL; 10 mmole). The mixture was then stirred in the ice bath over 10 h eventually coming to room temperature. The mixture was then filtered through a Buchner funnel and the organic phase was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate, brine and 1 M aqueous HCl. The organic phase was dried (MgSO_4), filtered and reduced in vacuo to provide 2.8g of a viscous oil (100%). $R_f=0.5$ (95:5, CHCl_3 ; MeOH).

Example 73Preparation of

25 [110]

The above oil was diluted with MeOH (70 mL) and 30 mL of a 1.5 M LiOH aqueous solution was added. The mixture was stirred at 25°C for 1 hour. The MeOH was then reduced in vacuo and the residue partitioned between ethyl ether and water. The aqueous phase was washed with ether (2 X 100 mL) and then the aqueous phase was neutralized to pH 1 with 1 M aqueous HCl. The

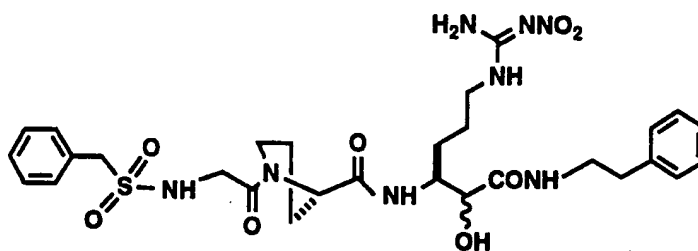
113

aqueous phase was then extracted with ethyl acetate (2 X 100 mL). The organic phase was then dried (MgSO₄), filtered and reduced in vacuo to provide 3.14 g (53%) of a tan solid. R_f=0.25 (70:30; trichloromethane: methanol).

Example 74

Preparation of

10 [111]



A 600 mg (1.1 mmole) portion of the product of Example 5 was taken up in trifluoroacetic acid at 0°C, and stirred for two hours. This solution was diluted with toluene (100 mL) and reduced in vacuo. This residue was dissolved in dimethylformamide (6 mL) and 361 mg (1.1 mmole) of the α -toluenesulfonylglycineproline acid of the previous example were added followed by 538 mg (1.22 mmole) of BOP and 1117 mg (11.0 mmole, 1.21 mL) of NMM and the solution was allowed to stir overnight.

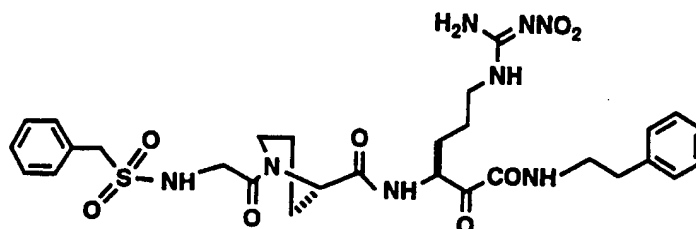
This solution was diluted in 50 mL 1 M HCl and extracted three times with ethyl acetate. The organics were combined and washed with water (three times), saturated sodium bicarbonate and brine. The solution was dried over magnesium sulfate and concentrated in vacuo to give 405 mg of the above compound as an orange/yellow foam. Thin layer chromatography showed no more starting material.

30

114

Example 75Preparation of

[112]

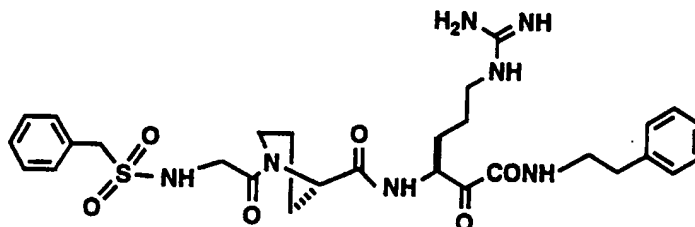


5

A 400 mg (0.605 mmole) portion of the product of the previous example, was taken up in 10 mL 1:1 toluene:DMSO with EDC. To this solution was added 312 mg (0.2 mL, 2.42 mmole, 4.0 eq) of dichloroacetic acid. This solution was stirred for one hour and ten minutes, diluted with 50 mL water and extracted twice with ethyl acetate (100 mL). The organics were combined, washed with brine, dried over magnesium sulfate, and concentrated in vacuo. This solution was purified on a (4:1:4 hexanes:methanol:dichloromethane) silica column to give 150 mg of the above compound as a clean white solid.

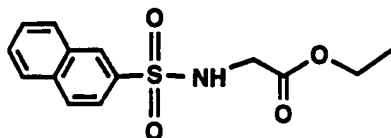
Example 76Preparation of

[113]



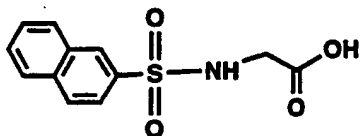
The product of the previous example was cleaved with hydrogen fluoride. The product was purified by HPLC to give the above compound which had an actual mass spectra peak (613.2) that correlated well with the expected value of 613.3.

115

Example 77Preparation of

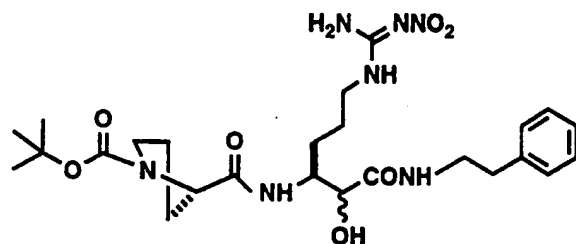
5 [114]

A 9.0 g (40 mmole) portion of 2-naphthyl-sulfonylchloride, a 5.6 g (40 mmole) portion of glycineethylesterhydrochloride salt, and a 14 mL (100
10 mmole) portion of triethylamine were mixed in tetrahydrofuran at 0°C to give a yield of 0.77 g of the above compound as a viscous oil.

Example 7815 Preparation of

[115]

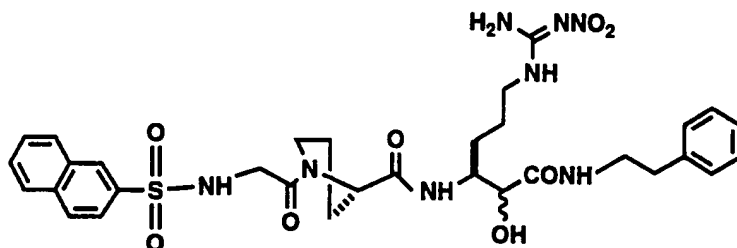
The product of the previous example was saponified
20 at room temperature with methanol/water/potassium hydroxide to give 6.41g of the above compound as a white solid.

Example 79Preparation of

5 [116]

A 3 g (6.63 mmole, 1.0 equivalent) portion of t-butoxycarbonylnitroarginine- α -hydroxy-2-phenethylamide was taken up in 15 mL of trifluoroacetic acid at 0°C and stirred for 1.5 hours. This solution was concentrated
10 twice with toluene in vacuo and taken up in dimethylformamide. To this solution were added 1427 mg (6.63 mmole, 1.0 equivalent) of t-butoxycarbonylproline acid, 7.3 mL (6705 mg, 66.3 mmole, 10 equivalents) of NMM and 3225 mg (7.3 mmole, 1.1 equivalents) of BOP. Thin layer
15 chromatography showed no reaction. More BOP and 4-methylmorpholine were added and thin layer chromatography showed some product was forming. This solution was allowed to stir overnight, diluted with 1 M HCl and extracted with ethyl acetate three times. The organics
20 were combined and washed with 1 M HCl, water (five times) saturated sodium bicarbonate, and brine; it was dried over magnesium sulfate, filtered and reduced in vacuo to provide the above compound.

117

Example 80Preparation of

5 [117]

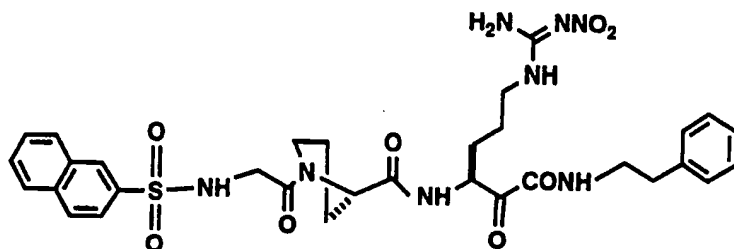
A 550 mg (1.0 mmole, 1.0 equivalent) portion of the product of the previous example was taken up in trifluoroacetic acid at 0°C and stirred for 1.5 hours.

10 This solution was concentrated twice with toluene in vacuo and taken up in dimethylformamide. To this solution were added 265 mg (1.0 mmole, 1.0 equivalents) of the product of Example 78 and 487 mg (1.1 mmole, 1.1 equivalents) of BOP. This solution was allowed to stir

15 overnight and turned dark brown. This solution was diluted with 1N HCl and extracted three times with ethyl acetate. The organics were combined, washed with 1N HCl, water (three times), saturated sodium bicarbonate, and brine, and dried over magnesium sulfate, filtered,

20 and reduced in vacuo to give 400 mg of the above compound as a white powder.

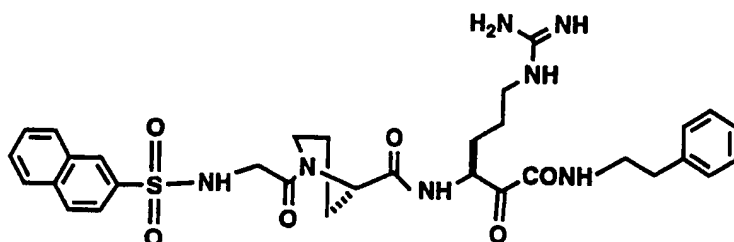
118

Example 81Preparation of

5 [118]

A 200 mg (0.300 mmole, 1.0 equivalent) portion of the alcohol of the previous example was taken up in 6 mL of 0.05 M 1:1 DMSO:PheMe. To this solution were added
10 577 mg (3.0 mmole, 10.0 equivalents) of EDC and 0.099 mL (155 mg, 1.2 mmole, 4.0 equivalents) of dichloroacetic acid. This solution was allowed to stir for 1.5 hours, diluted with water and extracted two times with ethyl acetate. The organics were combined, washed with brine,
15 dried over magnesium sulfate, and concentrated in vacuo. This solution was purified using a 4:1:4 hexanes: methanol:dichloromethane eluent on a silica column to give good separation. This gave 118 mg of the above compound that was stored in the freezer under nitrogen.

20

Example 82Preparation of

25 [119]

After hydrogen fluoride cleavage of the product of the previous example the molecular weight was found to

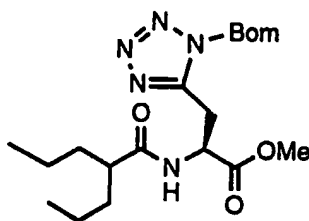
119

be 649.4, in good agreement with the expected value of 649.3.

Example 83

5 Preparation of:

3-(N-2-benzyloxymethyl)tetrazolyl-2-(2-propylpenta-
noylamido)propionic acid, methyl ester



[120]

2.0 g of 2-propylpentanoic acid is taken up in 10 mL of oxalyl chloride and this mixture is stirred overnight at 23°C under nitrogen. After this time, 100 mL of dry toluene is added and the volatiles removed in vacuo to yield the acid chloride which is used as indicated below.

1.0 g (2.5 mmole, 1 equiv.) of 3-(N-3-benzyloxymethyl)tetrazolyl-2-(1,1-dimethylethoxy)methan-amido-propionic acid, methyl ester is taken up in 10 mL of trifluoroacetic acid at -5°C and this solution stirred for 0.5 hours followed by concentration in vacuo.

The crude trifluoroacetate salt is taken up in toluene and this concentrated again to remove any residual trifluoroacetic acid. The crude trifluoroacetate salt is then taken up in 5 mL of dry tetrahydrofuran and 0.62 g (3.8 mmole, 1.5 equiv.) of 2-propylpentanoyl chloride, prepared as indicated above, is added followed by the addition of 1.07 mL of triethylamine. The reaction mixture is stirred for 2 hours at 23°C and diluted with 50 mL of ethyl acetate. The organics are washed with

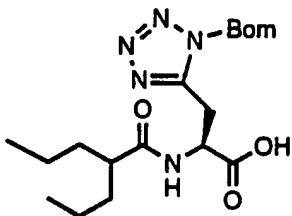
120

0.5 M HCl (2 x 25 mL), saturated sodium bicarbonate (25 mL), brine (25 mL), and dried over sodium sulfate. After decantation, the organics are concentrated in vacuo and purified by chromatography on silica (ethyl acetate/hexane eluent).

Example 84

Preparation of:

10 3-(N-2-benzyloxymethyl)tetrazolyl-2-(2-propylpentanoylamido)propionic acid



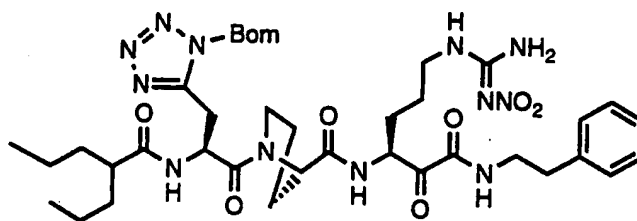
[121]

15

A 0.15 molar solution of 3-(N-2-benzyloxymethyl)-tetrazolyl-2-(2-propylpentanoylamido)propionic acid, methyl ester in methanol is prepared and 1.5 equivalents of a 1 M lithium hydroxide (aq.) is added. The reaction mixture is stirred until no starting material remains by thin layer chromatography (about 3 hours) and passed through Dowex 50x8-400 ion exchange resin and the resin washed with four column volumes of 1:1 methanol:water. The filtrate concentrated in vacuo to yield product in quantitative yield.

Example 85Preparation of:

3-(N-2-benzyloxymethyl)tetrazolyl-2-(2-propylpenta-
5 novlamido)propionoyl-L-Prolyl-L-N^g-nitro-arginine, 2-
phenethylketoamide



[122]

10

905 mg (2.25 mmole, 1 equiv.) of 3-(N-2-benzyloxy-
methyl)tetrazolyl-2-(2-propylpentanoylamido)propionic
acid, 1.56 g of L-prolyl-L-N^g-nitro-arginol, 2-
phenethylketoamide, trifluoroacetate salt (2.25 mmole, 1
15 equiv.), 1.19 g of BOP reagent (2.25 mmole, 1 equiv.),
and 34 mg of HOBt (0.2 mmole, 0.1 equiv.) were combined
in a 100 mL round bottom flask and 9 mL of dimethyl-
formamide was added followed by the addition of 1.48 mL
(13.5 mmole, 6 equiv.) of N-methylmorpholine. The reac-
20 tion mixture was stirred at room temperature for 3 hours
and then poured into a separatory funnel containing 100
mL of ethyl acetate and 10 mL of 3 M HCl. The organics
were washed with an additional 10 mL of 0.5 M HCl. The
aqueous washes were combined and back extracted with 20
25 mL of ethyl acetate. The organics were combined and
washed with 10 mL of 1 M sodium hydroxide followed by 10
mL of brine. After drying over sodium sulfate the
organics were filtered and concentrated in vacuo to
yield 2.7 g of crude product.

30 This was taken up in 20 mL of DMSO and 20 mL of
toluene. 4.32 g (22.5 mmole, 10 equiv.) of EDC (water
soluble carbodiimide) was added followed by the dropwise
addition of 0.75 mL of dichloroacetic acid. This was

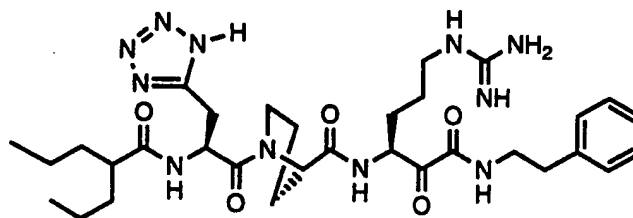
122

stirred at room temperature for 45 minutes. The solution was then poured into a separatory funnel containing 360 mL of ethyl acetate and 40 mL of water. The organics were washed with 50 mL of 0.5 M HCl followed by 50 mL of brine. After drying over sodium sulfate the organics were filtered, concentrated in vacuo, and immediately chromatographed on silica (2% - 10% methanol/methylene chloride gradient) to yield 1.59 g of product.

10 Example 86Preparation of:

3-Tetrazolyl-2-(2-propylpentanovlamido)propionoyl-L-Prolyl-L-arginine, 2-phenethylketoamide

15



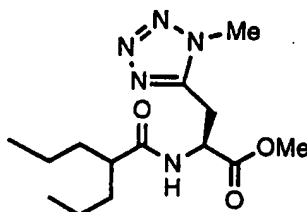
[42]

The product of the previous example was cleaved with hydrogen fluoride and purified to give the above compound.

20

Example 87Preparation of:

25 3-(N-2-Methyl)tetrazolyl-2-(2-propylpentanovlamido)propionic acid, methyl ester



[123]

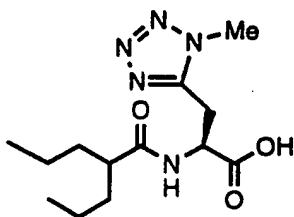
2.0 g of 2-propylpentanoic acid is taken up in 10 mL of oxalyl chloride and this mixture is stirred overnight at 23°C under nitrogen. After this time, 100 mL of dry toluene is added and the volatiles removed in vacuo to yield the acid chloride which is used as indicated below. 1.0 g (2.5 mmole, 1 equiv.) of 3-(N-3-benzyloxymethyl)tetrazolyl-2-(1,1-dimethylethoxy)methan-amido-propionic acid, methyl ester is taken up in 10 mL of trifluoroacetic acid at -5°C and this solution stirred for 0.5 hours followed by concentration in vacuo. The crude trifluoroacetate salt is taken up in toluene and this concentrated again to remove any residual trifluoroacetic acid.

The crude trifluoroacetate salt is then taken up in 5 mL of dry tetrahydrofuran and 0.62 g (3.8 mmole, 1.5 equiv.) of 2-propylpentanoyl chloride, prepared as indicated above, is added followed by the addition of 1.07 mL of triethylamine. The reaction mixture is stirred for 2 hours at 23°C and diluted with 50 mL of ethyl acetate. The organics are washed with 0.5 M HCl (2 x 25 mL), saturated sodium bicarbonate (25 mL), brine (25 mL), and dried over sodium sulfate. After decantation, the organics are concentrated in vacuo and purified by chromatography on silica (ethyl acetate/hexane eluent).

Example 88

Preparation of:

3-(N-2-methyl)tetrazolyl-2-(2-propylpentanoylamido)propionic acid



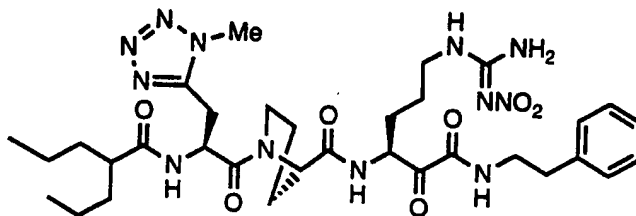
[124]

124

A 0.15 molar solution of 3-(N-2-benzyloxy-methyl)tetrazolyl-2-(2-propylpentanoylamido)propionic acid, methyl ester in methanol is prepared and 1.5 equivalents of a 1 M lithium hydroxide (aq.) is added. The reaction mixture is stirred until no starting material remains by thin layer chromatography (about 3 hours) and passed through Dowex 50x8-400 ion exchange resin and the resin washed with four column volumes of 1:1 methanol:water. The filtrate concentrated in vacuo to yield product in quantitative yield.

Example 89Preparation of:

3-(N-2-methyl)tetrazolyl-2-(2-propylpentanoyl-amido)propionoyl-L-Prolyl-L-N^o-nitro-arginine, 2-phenethylketoamide



[125]

20

410 mg (1.44 mmole, 1 equiv.) of 3-(N-2-methyl)tetra-zolyl-2-(2-propylpentanoylamido)propionic acid, 834 mg (1.44 mmole, 1 equiv.) of L-prolyl-L-N^o-nitro-argininol, 2-phenethylketoamide, trifluoroacetate salt, 636 mg (1.44 mmole, 1 equiv.) of BOP, and 34 mg (0.14 mmole, 0.1 equiv.) of HOBT were combined in a 100 mL round bottom flask and 7 mL of dimethylformamide was added followed by the addition of 0.948 mL (8.63 mmole, 6 equiv.) of N-methylmorpholine. The reaction mixture was stirred at room temperature for 3 hours and then poured into a separatory funnel containing 90 mL of ethyl acetate and 10 mL of 3 M HCl. The organics were washed with an additional 10 mL of 0.5 M HCl. The

aqueous washes were combined and back extracted with 20 mL of ethyl acetate. The organics were combined and washed with 10 mL of 1 M sodium hydroxide followed by 10 mL of brine. After drying over sodium sulfate the
5 organics were filtered and concentrated in vacuo to yield 1.67 g of crude product.

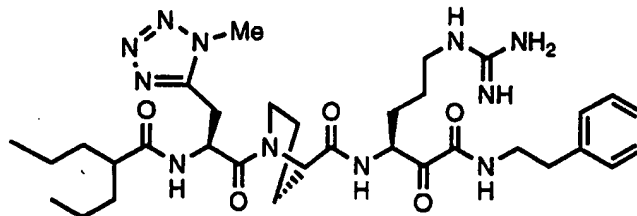
This was taken up in 20 mL of DMSO and 20 mL of toluene. 4.32 g (22.5 mmole, 10 equiv.) of EDC (water soluble carbodiimide) was added followed by the dropwise
10 addition of 0.75 mL of dichloroacetic acid. This was stirred at room temperature for 45 minutes. The solution was then poured into a separatory funnel containing 360 mL of ethyl acetate and 40 mL of water. The organics were washed with 50 mL of 0.5 M HCl followed by
15 50 mL of brine. After drying over sodium sulfate the organics were filtered, concentrated in vacuo, and immediately chromatographed on silica (2% - 10% methanol/methylene chloride gradient) to yield 463 mg of product.

20

Example 90Preparation of:

3-(N-2-methyl)tetrazolyl-2-(2-propylpentanovlamido)pro-
25 pionovyl-L-Prolvl-L-arginine, 2-phenethylketoamide

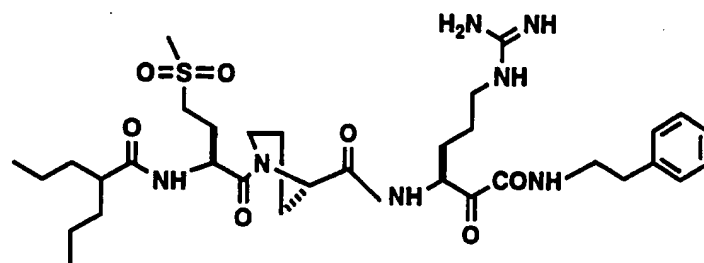
[43]



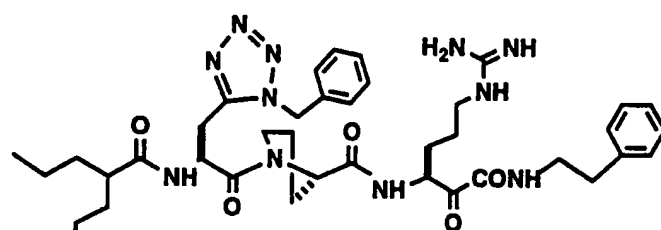
The product of the previous example was cleaved
30 with hydrogen fluoride to give the above compound.

The following compounds can also be made by those skilled in the art using the methods of the present invention.

126

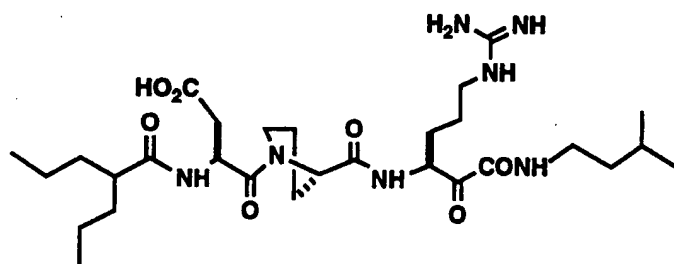


[39]



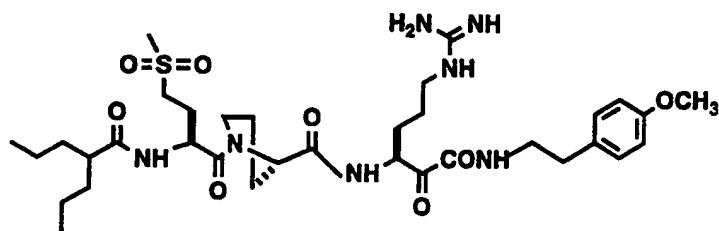
5

[126]



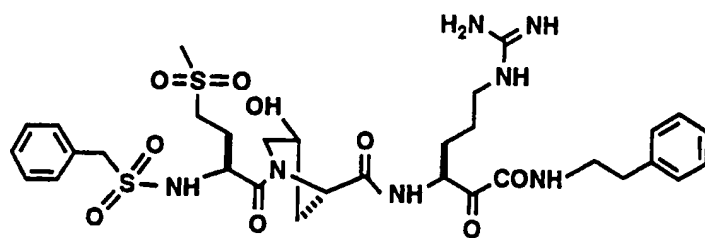
[127]

10

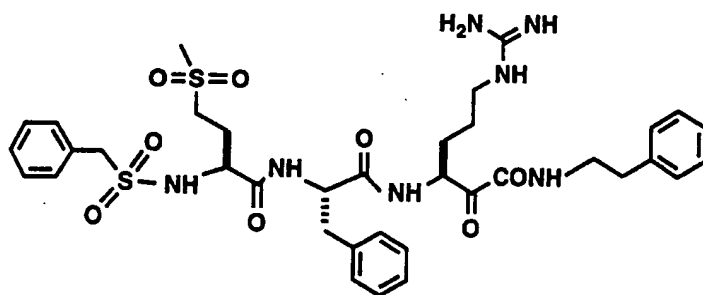


[40]

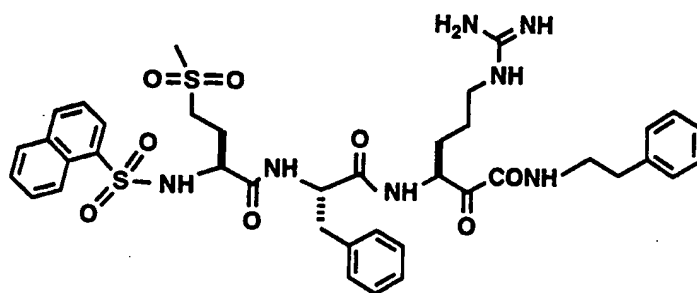
127



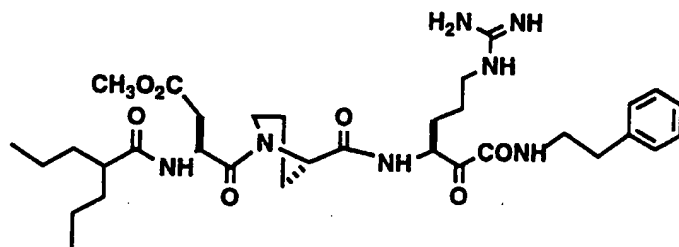
[41]



5 [128]



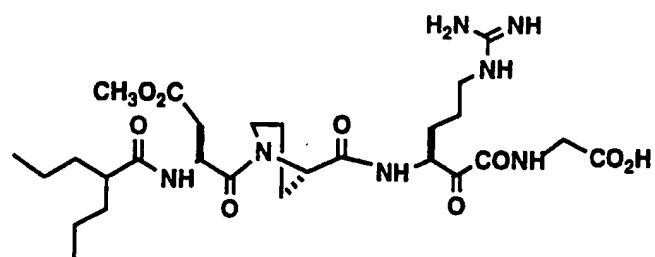
[129]



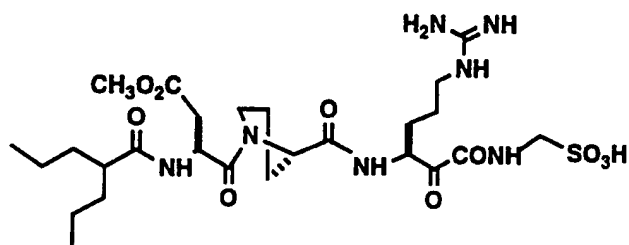
10

[38]

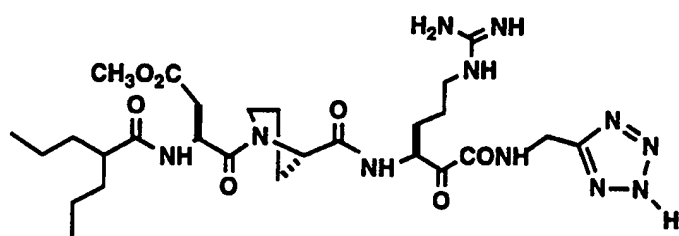
128



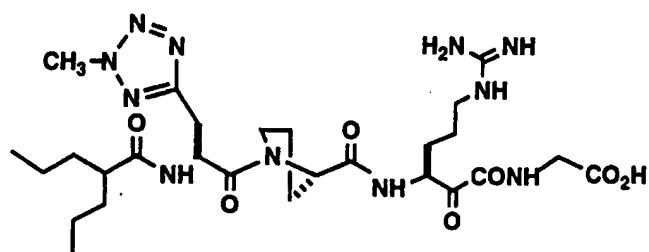
[6]



5 [7]



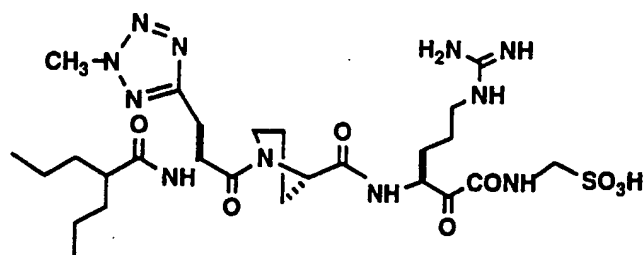
[8]



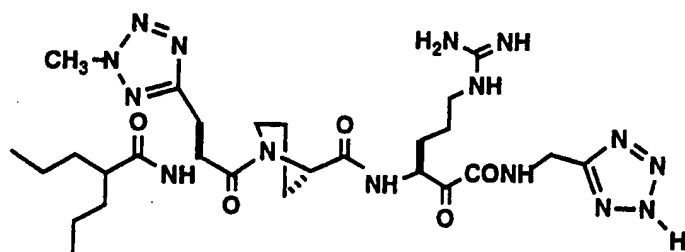
10

[9]

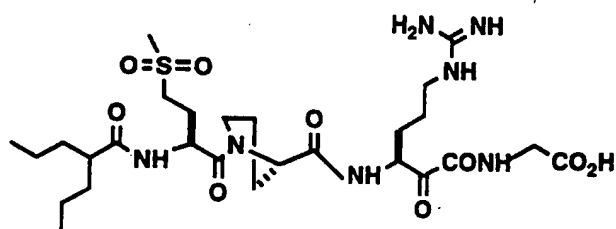
129



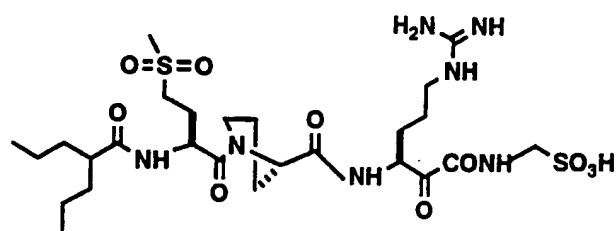
[10]



5 [11]

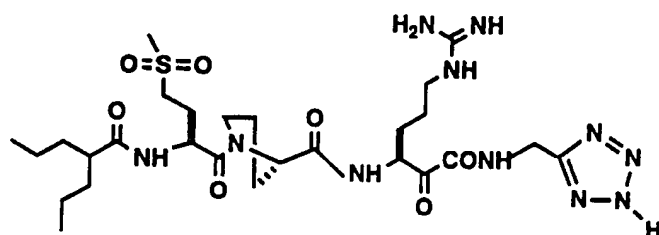


[12]



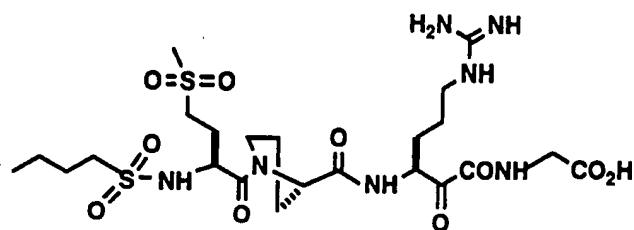
10

[13]

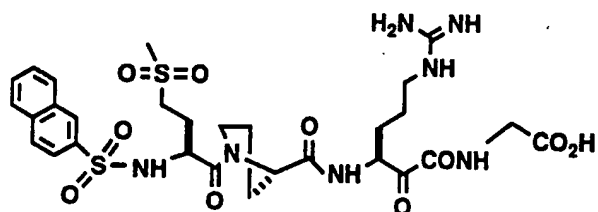


[14]

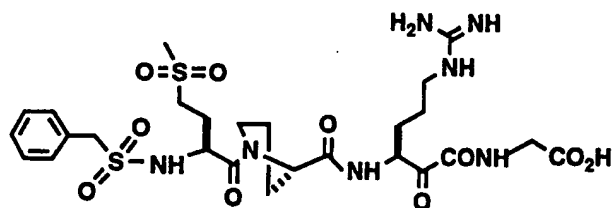
130



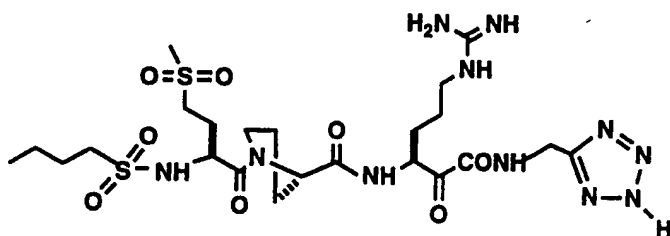
[15]



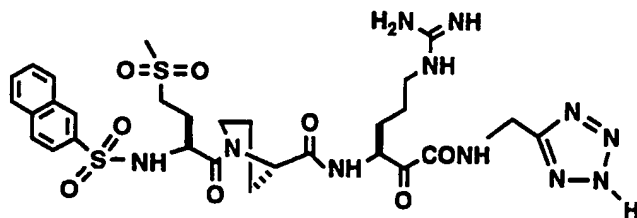
[16]



[17]

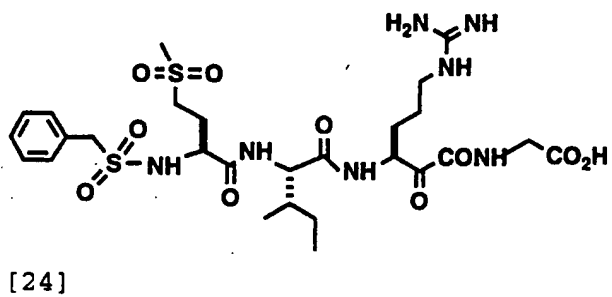
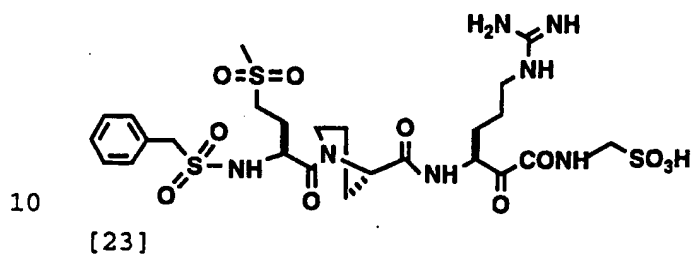
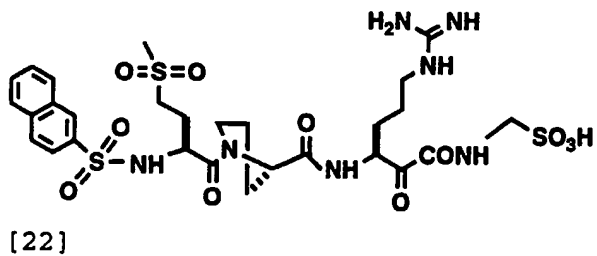
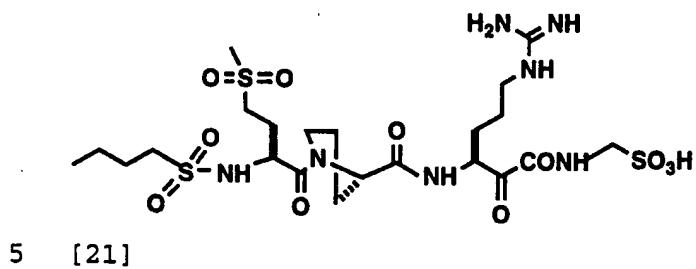
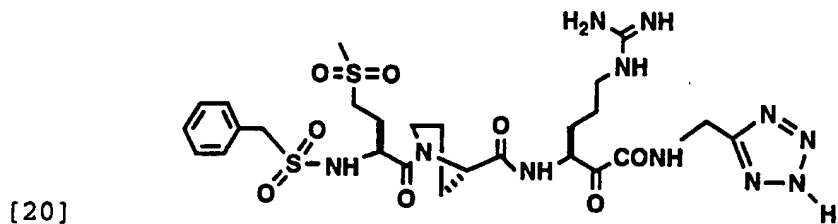


[18]



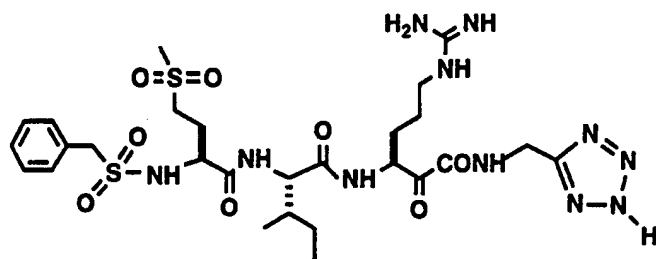
[19]

131

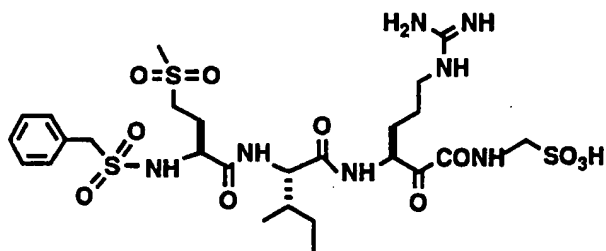


15

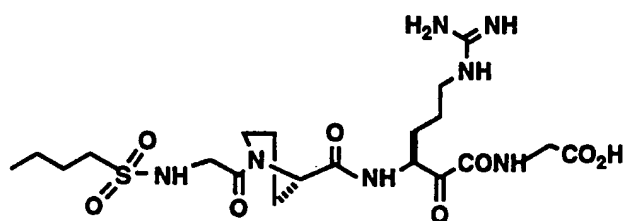
132



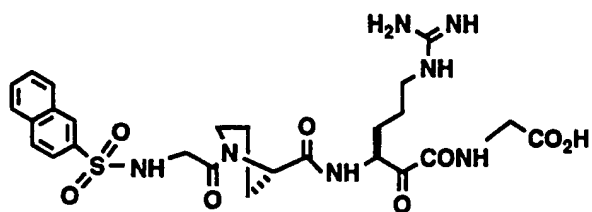
[25]



5 [26]

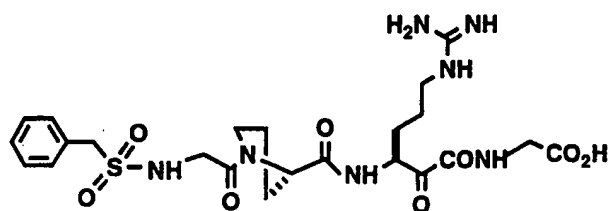


[27]



10

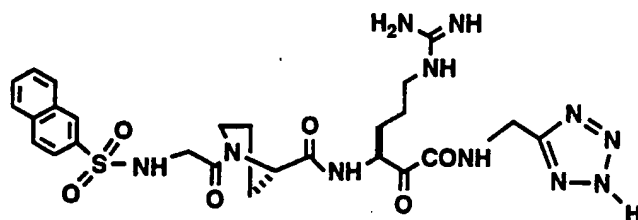
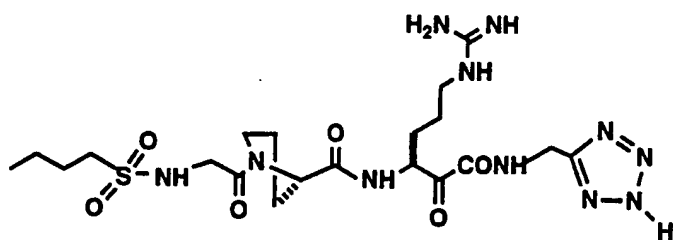
[28]



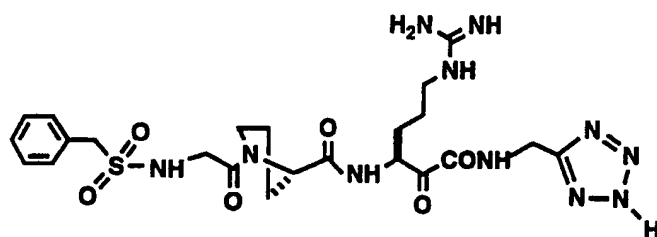
[29]

15

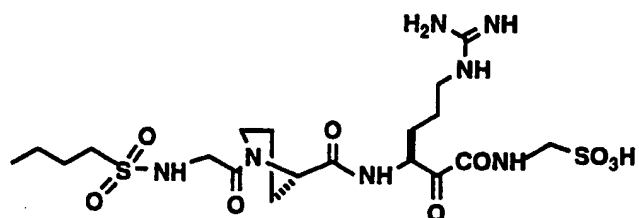
133



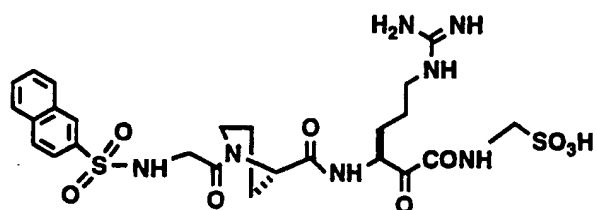
5



[32]

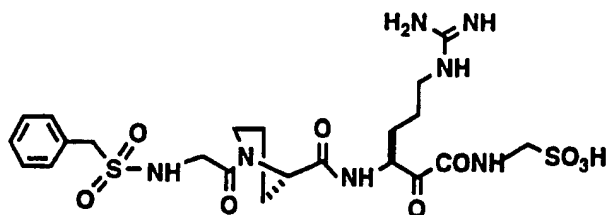


10



15

134



[35]

Example A5 Thrombin Assay

The ability of the compounds of the present invention to act as inhibitors of thrombin catalytic activity was assessed by determining their inhibition constant, K_i , and the concentration which inhibited enzyme activity by 50%, IC_{50} , against thrombin.

Enzyme activity was determined using the chromogenic substrates, S2266 (H-D-valyl-L-leucyl-L-arginine-p-nitroaniline, obtained from Kabi Diagnostica) or Pefachrome t-PA (CH_3SO_2 -D-hexahydrotyrosine-glycyl-L-Arginine-p-nitroaniline, obtained from Pentapharm Ltd.). The substrates were reconstituted in deionized water prior to use. Purified human α -thrombin was obtained from Enzyme Research Laboratories, Inc. The buffer used for all assays was HBSA (10 mM HEPES, pH 7.5, 150 mM sodium chloride, 0.1% bovine serum albumin).

The assay for K_i determinations was conducted by combining in appropriate wells of a Corning microtiter plate, 50 μ L of HBSA, 50 μ L of the test compound at a specified concentration diluted in HBSA (or HBSA alone for V_o (uninhibited velocity) measurement), and 50 μ L of the chromogenic substrate S-2266 at a specified concentration diluted in HBSA. At time zero, 50 μ L of α -thrombin diluted in HBSA, was added to the wells yielding a final concentration of 0.5 nM in a total volume of 200 μ L. Velocities of S-2266 substrate hydrolysis which occurred over a designated time period was measured by the change in absorbance at 405nm using a Thermo Max[®] Kinetic Microplate Reader.

Ki values were determined for test compounds using the following methodologies: 1) For test compounds exhibiting slow binding or slow-tight binding kinetics, Ki values were determined using the relationships developed by Williams and Morrison, Methods in Enzymology, 63: 437 (1979) using steady state velocities (Vs) measured over 40 minutes. The extent of substrate hydrolysis was less than 5% over the course of this assay. 2) For test compounds showing rapid, reversible kinetics of inhibition, Ki values were determined from initial velocities using the relationships developed by Dixon, M., Biochem. J., 129: 197 (1972).

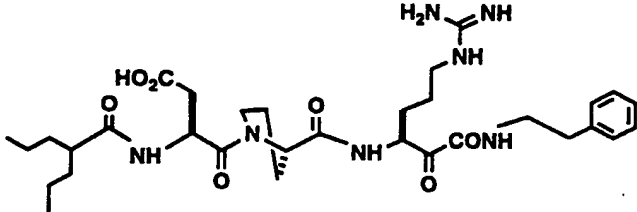
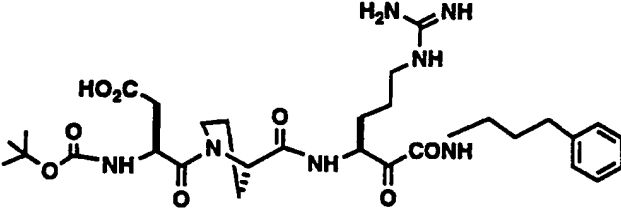
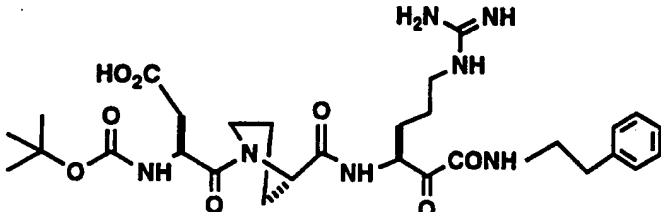
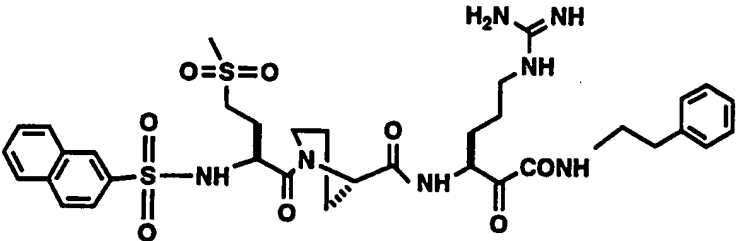
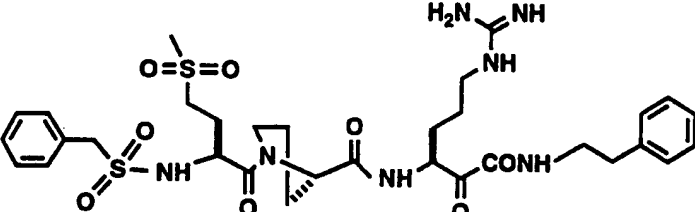
IC50 determinations were conducted where HBSA (50 mL), α -thrombin (50 μ l) and inhibitor (50 μ l) (covering a broad concentration range), were combined in appropriate wells and incubated for 30 minutes at room temperature prior to the addition of substrate Pefachrome-t-PA (50 μ l). The initial velocity of Pefachrome t-PA hydrolysis was measured by the change in absorbance at 405nm using a Thermo Max® Kinetic Microplate Reader over a 5 minute period in which less than 5% of the added substrate was utilized. The concentration of added inhibitor which caused a 50% decrease in the initial rate of hydrolysis was defined as the IC50 value.

Table I below gives the Ki and IC50 values for selected test compounds. The data shows their utility as potent in vitro inhibitors of human α -thrombin.

136

Table I. Inhibitor Constants (K_i) and IC₅₀'s of Compounds

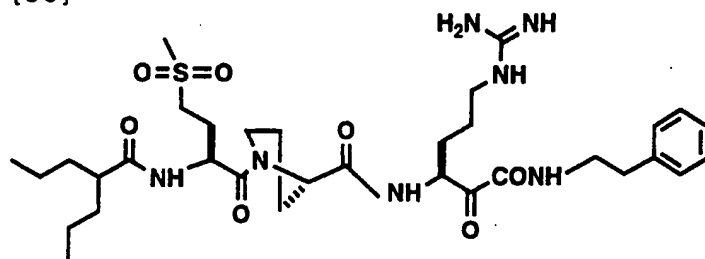
Compound K_i (nM) IC₅₀ (nM)

5	 <p>[4]</p>	1.5	0.7
10	 <p>[5]</p>	5.5	6.5
	 <p>[3]</p>	11.0	2.2
	 <p>[37]</p>	---	0.42
15		---	0.37

SUBSTITUTE SHEET

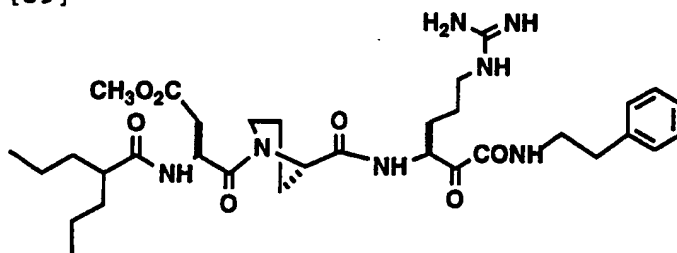
137

[36]



0.09 0.36

[39]



0.06 0.48

5 [38]

Example B

Experimental Models of Thrombosis

The antithrombotic properties of the compound of
10 Example 8 was evaluated using the following established
experimental models of acute thrombosis.

Extracorporeal Shunt Model in Rats

This is one of the most common and generally used models in the evaluation of antithrombotic compounds. Smith, J.R. and White, A.M. *Br. J. Pharmacol.*, 77: 29-38 (1982). In this model a localized clot made up of primarily fibrin with some platelet and macrophage involvement (Shand, R. A. and Smith, J.R. and Wallis, R. B. *Thromb. Res.*, 36: 223-232 (1984)), is formed on an artificial thrombogenic surface (typically a segment of silk or cotton thread) contained in a sialstic chamber which is part of an exteriorized shunt between the carotid artery and jugular vein.

25 The effect of the compound of Example 8 on the formation of a thrombus on the thrombogenic surface was

measured using clot weight as the primary end point in the model.

Male Harlan Sprague Dawley rats (420-450 g) were acclimated at least 72 hours prior to use. The animals were fasted for 12 hours prior to surgery with free access to water. The animals were anesthetized with a sodium pentobarbital (Nembutal) given intraperitoneally at a dose of 50 mg/kg body weight and placed on a isothermal pad to maintain body temperature. The level of anesthesia was monitored every 15 minutes by: neuro-response to a tail pinch, respiration and core temperature. The desired depth of surgical anesthesia was maintained by administering subsequent doses (5 mg/kg) intravenously. The left femoral artery was catheterized using standard procedures for blood pressure monitoring and blood sampling, with polyethylene tubing (PE50). The left and right femoral veins were catheterized with PE50 tubing for delivery of anesthetic and test compounds, respectively.

Following surgery the animals were randomized in either a control (saline infusion) or treatment group (Compound of Example 8) with at least 6 animals per group per dose. The exteriorized shunt was assembled prior to catheterization by connecting two pieces of saline filled 12.5 cm PE90 tubing with a 6 cm piece of PE160 tubing containing a 6 cm piece of silk suture size 3 and clamped with hemostats. A small 0.5 cm portion of the silk thread protrudes from the junction of the chamber with the shunt. The left jugular vein and right carotid artery were catheterized with the ends of the PE90 shunt. Prior to unclamping the shunt, the test compound (Compound of Example 8) was dissolved in normal saline, and infused via the right femoral vein as an initial bolus (0.5 mg/kg) followed by a continuous intravenous infusion (at the designated doses shown in the following table) for 30 minutes prior to exposure of the suture to flowing blood. Blood pressure, heart rate

core temperature and respiration were monitored continuously. At the designated time, blood flow through the chamber was initiated by unclamping the shunt and allowed to flow for a period of 15 minutes during which time the test compound continued to be administered. At the end of the exposure period both sides of the chamber were clamped and the suture containing the clot removed following detachment of the arterial end of the chamber. The clot was immediately weighed and recorded. Following termination of the experiment the animal was euthanized with a 120 mg/kg dose of Nembutal. One experiment was performed per animal.

The efficacy of the compound of Example 8 as an antithrombotic agent in this *in vivo* model was demonstrated by the reduction in clot size, as shown in Table II below.

Table II. Efficacy of the Compound of Example 8 in Rat Extracorporeal Shunt Model.

20	<u>Treatment Group</u>	<u>Clot size (mg)^a</u>
	Control	41.30±3.42
	Group1	38.37±4.49
25	Group2	17.22±1.79 *
	Group3	10.20±0.636 *
30	Control-no treatment	
	Group1-0.5mg/kg i.v. bolus+20mg/kg/min i.v. infusion	
	Group2-0.5mg/kg i.v. bolus+50mg/kg/min i.v. infusion	
	Group3-0.5mg/kg i.v. bolus+100mg/kg/min i.v. infusion	

α -weights are designated as the mean \pm S.E.M. (n=6).

*- $p \leq 0.01$ vs Control by one-way ANOVA followed by Newman-Kuels Test.

Rat model of FeCl₃-induced platelet-dependent arterial thrombosis

This is a well characterized model of platelet dependent, arterial thrombosis which has been used in the evaluation potential antithrombotic compounds such as direct thrombin inhibitors. Kurz, K. D., Main, B. W., and Sandusky, G. E., *Thromb. Res.*, 60: 269-280 (1990). In contrast to the exteriorized shunt model, thrombus development in this model is relatively heparin insensitive which suggests that this model may be more representative of the type of thrombosis which has been observed clinically in newly re-canalized coronary vessels following balloon angioplasty or enzymatic thrombolysis. In this model a platelet-rich, occlusive thrombus is formed in a segment of the rat carotid artery treated with a fresh solution of FeCl₃ absorbed to a piece of filter paper. The FeCl₃ is thought to diffuse into the treated segment of artery and causes de-endothelialization resulting in thrombus formation. The effect of a test compound on the incidence of occlusive thrombus formation following the application of the FeCl₃ is monitored by ultrasonic flowtometry and is used as the primary end point. The use of flowtometry is a modification of the original procedure in which thermal detection of clot formation was employed. Kurz, K. D., Main, B. W., and Sandusky, G. E., *Thromb. Res.*, 60: 269-280 (1990).

Male Harlan Sprague Dawley rats (420-450 g) were acclimated at least 72 hours prior to use and fasted for 12 hours prior to surgery with free access to water. The animals were prepared, anesthetized with Nembutal with catheters for blood pressure monitoring, drug and anesthesia delivery being implanted as described above. The left carotid artery was isolated by making a midline cervical incision followed by blunt dissection and spreading techniques to separate a 2 cm segment of the vessel from the carotid sheath. A silk suture is

inserted under the proximal and distal ends of the isolated vessel to provide clearance for the placement of a ultrasonic flow probe (Transonic) around the proximal end of the vessel. The probe is then secured with a stationary arm.

Following surgery the animals were randomized in either a control (saline infusion) or treatment group with test compound (Compound of Example 8) with at least 6 animals per group per dose. The test compounds were administered as described above after placement of the flow probe and stabilization of the preparation for a period of 30 min prior to the thrombogenic stimulus. At $t=0$, a 3mm diameter piece of filter paper (Whatman #3) soaked with 10 mL of a 35% solution of fresh FeCl_3 (made up in water) was applied the segment of isolated carotid artery distal to the flow probe. Blood pressure, blood flow, heart rate, and respiration were monitored for 60 minutes.

The incidence of occlusion (defined as the attainment of zero blood flow) was recorded as the primary end point. Following the 60 minute observation period the flow probe was removed and the area cleared of all excess fluid. The distal and proximal sutures were tied off and arterial clamps placed on the far proximal and distal ends of the segment. The isolated segment was cut out, blotted dry on filter paper and weighed. The segment was re-weighed following removal of the clot and the difference recorded as total % clot weight. The animals were euthanized as described above.

The efficacy of the compound of Example 8 as an antithrombotic agent in this in vivo model was demonstrated by the reduction in the incidence of occlusion and in clot size, as shown in Table III below.

142

Table III. Results of the Compound of Example 8 in the FeCl₃ Model of Thrombosis in Rats.

5	<u>Treatment Group^a</u>	<u>Incidence of Occlusion^b</u>	<u>Clot Size^c</u>
	Control	6/6	68.65±3.75
	Group1	5/6	40.73±8.0***
	Group2	1/6 *	12.56±5.96***
	Group3	0/6 **	4.46±3.49***

10

a- Control-no treatment

Group1-0.5mg/kg i.v. bolus+20mg/kg/min i.v. infusion

Group2-0.5mg/kg i.v. bolus+50mg/kg/min i.v. infusion

Group3-0.5mg/kg i.v. bolus+100mg/kg/min i.v. infusion

15

b-Occlusion is defined as the establishment of zero blood flow through the treated segment of the carotid artery.

20 c-Clot size is defined as: [Isolated clot/(Intact segment-Empty segment)] X 100. Numbers represent the mean ± S.E.M. (n=6).

*p≤0.05 vs Control by Chi-Square Analysis

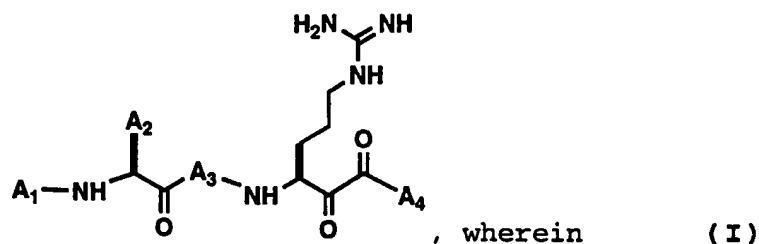
25 **p≤0.005 vs Control by Chi-Square Analysis

***p<0.01 vs Control by one-way ANOVA followed by Newman-Kuels Test

30 These in vivo data clearly demonstrated the antithrombotic efficacy of the Compound of Example 8 in two well established models of experimental thrombosis.

Claims

1. A compound of the formula



5 (a) A₁ is selected from the group consisting of
 R₁-C(O)-, R₁-O-C(O)-, R₁-NH-C(O)-, R₁-S(O₂)-, R₁-O-S(O₂)-, and

R₁-NH-S(O₂)-, wherein R₁ is selected from the group consisting of

10 alkyl of 1 to about 12 carbon atoms,
 alkenyl of about 3 to about 6 carbon atoms,
 aryl of about 6 to about 14 carbon atoms which
 is optionally mono-substituted with X₁ or optionally di-substituted with X₁ and X₂,

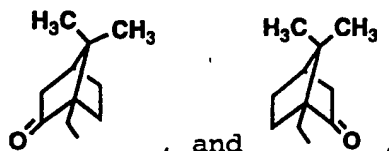
15 aralkyl of about 6 to about 15 carbon atoms
 which is optionally mono-substituted in the aryl ring
 with X₁ or optionally di-substituted in the aryl ring
 with X₁ and X₂,

20 aralkenyl of about 8 to about 15 carbon atoms
 which is optionally mono-substituted in the aryl ring
 with X₁ or optionally di-substituted in the aryl ring
 with X₁ and X₂,

perfluoroalkyl of 1 to about 12 carbon atoms,
 perfluoroaryl of about 6 to about 14 carbon

25 atoms,

trimethylsilylalkyl of 4 to about 8 carbon
 atoms,



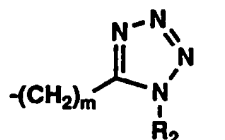
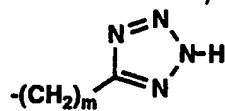
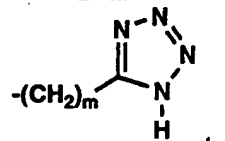
wherein X₁ and X₂ are independently selected from the
 30 group consisting of bromo, chloro, fluoro, Y₁-, HO-, Y₁-

144

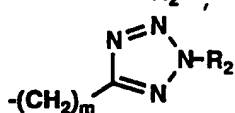
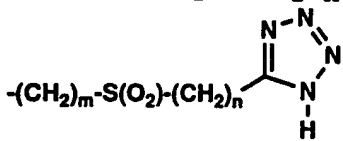
O-, NH₂-, Y₁-NH-, (Y₁,Y₂)N-, Y₁-C(O)-NH-, HS-, Y₁-S-,
 Y₁-S(O)-, Y₁-S(O₂)-, HO-S(O₂)-, Y₁-O-S(O₂)-, NH₂-S(O₂)-
 5 and Y₁-NH-S(O₂)-, wherein Y₁ and Y₂ are independently
 selected from the group consisting of trifluomethyl,
 pentafluoroethyl, alkyl of 1 to about 12 carbon atoms,
 aryl of about 6 to about 14 carbon atoms, and aralkyl of
 about 6 to about 15 carbon atoms;

(b) A₂ is selected from the group consisting of
 hydrogen,

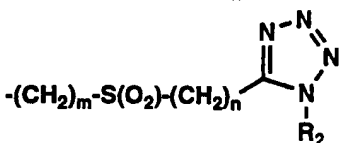
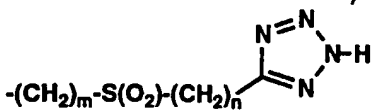
10

R₂-,-(CH₂)_m-C(O)-O-H,-(CH₂)_m-C(O)-O-R₂,

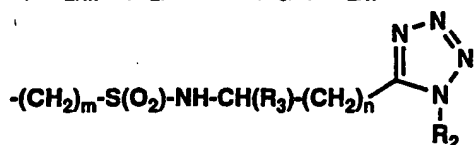
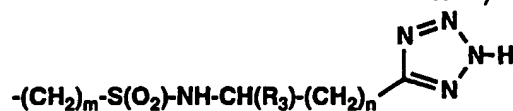
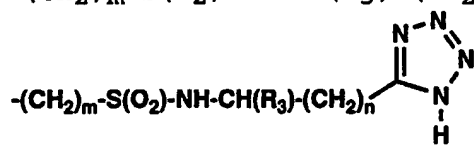
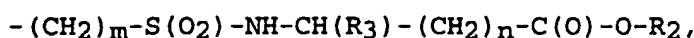
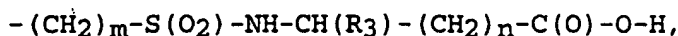
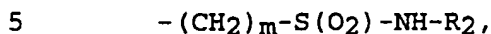
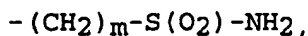
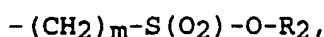
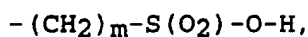
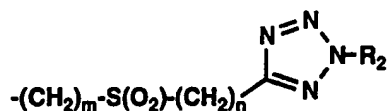
15

-(CH₂)_m-S(O₂)-R₂,-(CH₂)_m-S(O₂)-(CH₂)_n-C(O)-OH,-(CH₂)_m-S(O₂)-(CH₂)_n-C(O)-O-R₂,

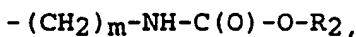
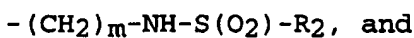
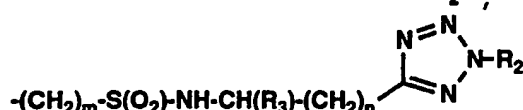
20



145



10



wherein

15 (i) m is 1, 2 or 3;

(ii) n is 0, 1, 2, 3 or 4;

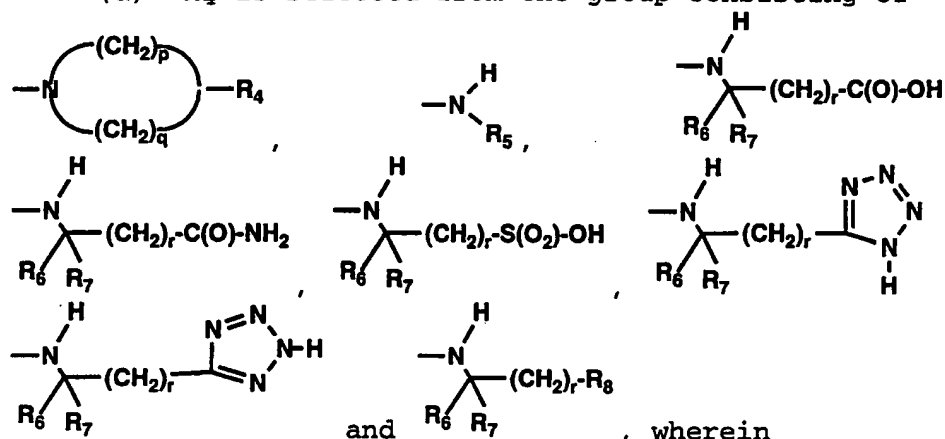
(iii) R₂ is selected from the group consisting of alkyl of 1 to about 12 carbon atoms, alkenyl of about 3 to about 6 carbon atoms, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms and aralkenyl of about 8 to about 15 carbons atoms; and

20 (iv) R₃ is selected from the group consisting of hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms, and alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the

group consisting of $-\text{OH}$, $-\text{C}(\text{O})-\text{OH}$, $-\text{C}(\text{O})-\text{NH}_2$, $-\text{S}-\text{CH}_3$, $-\text{S}(\text{O})-\text{CH}_3$, $-\text{S}(\text{O}_2)-\text{CH}_3$ and $-\text{NH}-\text{S}(\text{O}_2)-\text{CH}_3$;

- (c) A_3 is an amino acid residue of the amino acid selected from the group consisting of L-alanine, L-azetidinecarboxylic acid, glycine, L-isoleucine, L-leucine, L-lysine mono-substituted at its ϵ -amino group with $\text{R}_2-\text{S}(\text{O}_2)-$, L-methionine sulfone, N-methylglycine, L-ornithine mono-substituted at its δ -amino group with $\text{R}_2-\text{S}(\text{O}_2)-$, L-pipecolic acid, L-phenylalanine, L-proline, L-valine, and trans-4-hydroxy-L-proline; and

(d) A_4 is selected from the group consisting of



- (i) p and q are each independently selected integers from 1 to 5 wherein the sum of $p + q$ is 4 to 8;
- (ii) R_4 is aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, alkoxy of 1 to about 4 carbon atoms, $-\text{NH}_2$, $-\text{C}(\text{O})-\text{OH}$, $-\text{C}(\text{O})-\text{NH}_2$, fluoro, $-\text{OH}$, $-\text{NO}_2$ and $-\text{CF}_3$;
- (iii) R_5 is aryl of about 6 to 14 carbon atoms;
- (iv) R_6 is selected from the group consisting of hydrogen and alkyl of 1 to about 4 carbon atoms;
- (v) R_7 is selected from the group consisting of hydrogen; alkyl of 1 to about 4 carbon atoms;

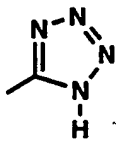
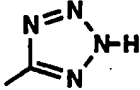
147

aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH₂, -C(O)-OH, -C(O)-NH₂, fluoro, -OH, -NO₂, -CF₃,
 5 alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms;

aralkyl of about 6 to about 15 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of
 10 -NH₂, -C(O)-OH, -C(O)-NH₂, fluoro, -OH, -NO₂, -CF₃, alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms; and

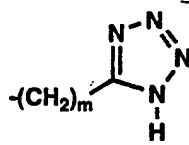
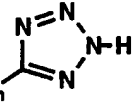
alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -OH, -C(O)-OH, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and
 15 -NH-S(O₂)-CH₃; and

(vi) R₈ is selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, and
 20 aralkyl of about 6 to about 15 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, wherein X₃ and X₄ are independently selected from the group consisting of -C(O)-OH,

25 -S(O₂)-OH,  and ; and

(vii) r is 0, 1, 2 or 3; and pharmaceutically acceptable salts thereof.

2. A compound of claim 1, wherein A₂ is selected
 30 from the group consisting of -(CH₂)_m-C(O)-O-

H,  and .

3. A compound of claim 2, wherein m is 1 or 2.

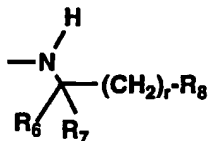
4. A compound of claim 3, wherein A₁ is R₁-C(O)-.

5. A compound of claim 4, wherein R₁ is selected from the group consisting of alkyl of 1 to about 12 carbon atoms.

6. A compound of claim 5, wherein R₁ is selected from the group consisting of 2,2-dimethylpropyl, 3-methylbutyl and 1-propylbutyl.

7. A compound of claim 6, wherein A₃ is L-proline.

8. A compound of claim 7, wherein A₄ is



9. A compound of claim 8, wherein R₆ and R₇ are each hydrogen.

10. A compound of claim 9, wherein r is 1.

11. A compound of claim 10, wherein R₈ is selected from the group consisting of alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, and aralkyl of about 6 to about 15 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄.

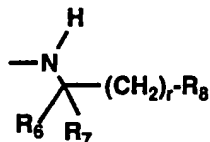
12. A compound of claim 11, wherein R₈ is selected from the group consisting of -CH(CH₃)₂, phenyl and benzyl.

13. A compound of claim 3 wherein A₁ is R₁-O-C(O)-.

14. A compound of claim 13, wherein R₁ is 1,1-dimethylethyl.

15. A compound of claim 14, wherein A₃ is L-proline.

16. A compound of claim 15, wherein A₄ is



17. A compound of claim 16, wherein R₆ and R₇ are each hydrogen.

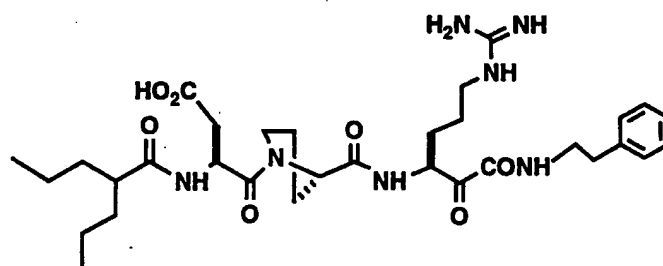
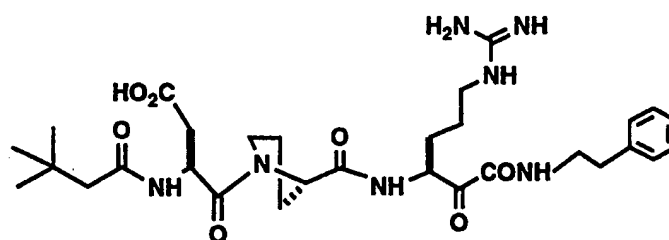
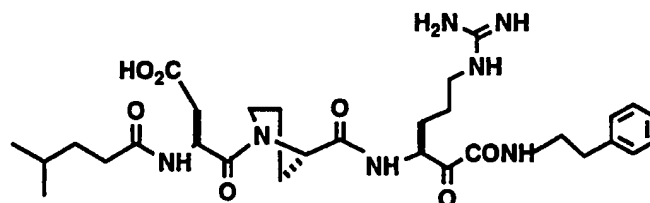
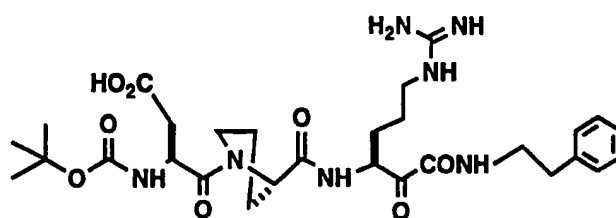
18. A compound of claim 17, wherein r is 1.

19. A compound of claim 18, wherein R₈ is selected from the group consisting of alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, and aralkyl of about 6 to about 15 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄.

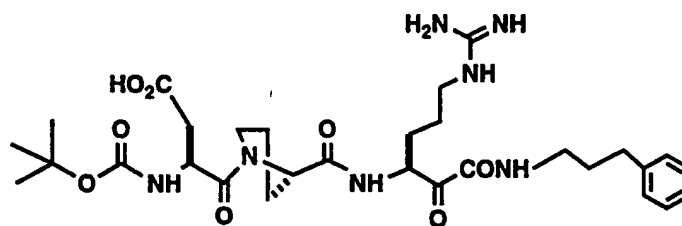
20. A compound of claim 19, wherein R₈ is selected from the group consisting of -CH(CH₃)₂, phenyl and benzyl.

21. A compound of claim 2 selected from the group consisting of

150

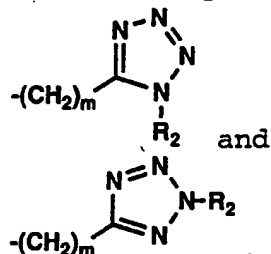


and



151

22. A compound of claim 1, wherein A₂ is selected from the group consisting of $-(CH_2)_m-C(O)-O-R_2$,



5

23. A compound of claim 22, wherein R₂ is methyl.

24. A compound of claim 23, wherein m is 1.

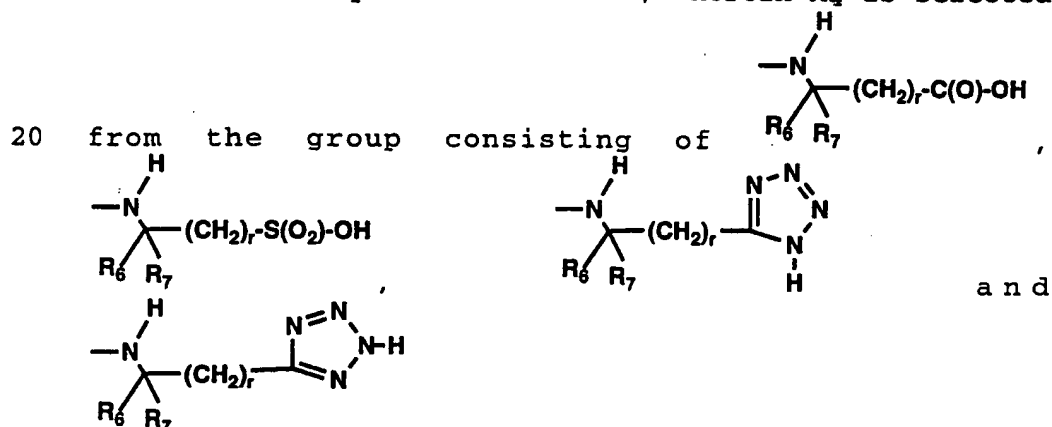
10 25. A compound of claim 24, wherein A₁ is R₁-C(O)-.

26. A compound of claim 25, wherein R₁ is selected from the group consisting of 2,2-dimethylpropyl, 3-methylbutyl, 1-propylbutyl and 2-phenylethyl.

15

27. A compound of claim 26, wherein A₃ is L-proline.

28. A compound of claim 27, wherein A₄ is selected



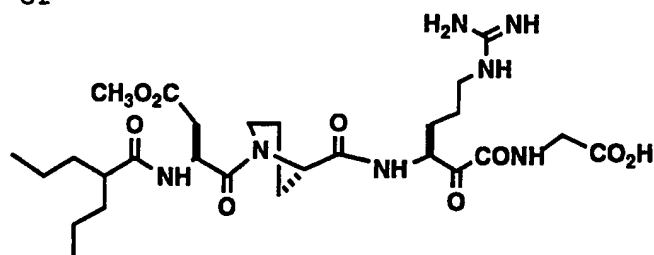
25 29. A compound of claim 28, wherein R₆ and R₇ are each hydrogen.

30. A compound of claim 29, wherein r is 0.

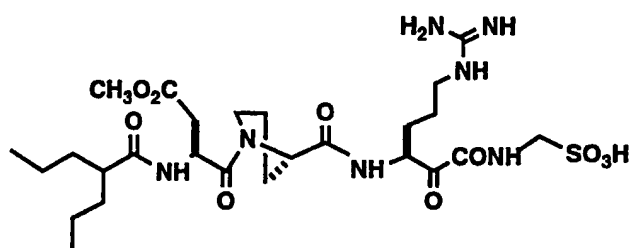
31. A compound of claim 22 selected from the group consisting of

5

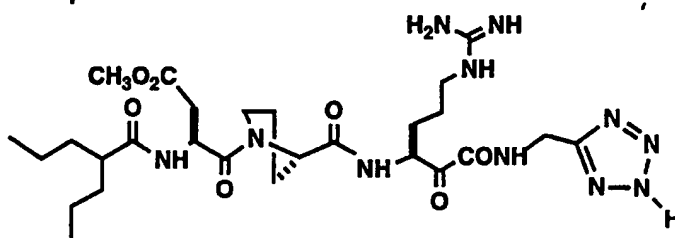
[6]



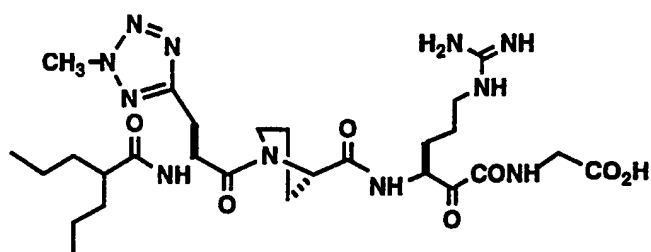
[7]



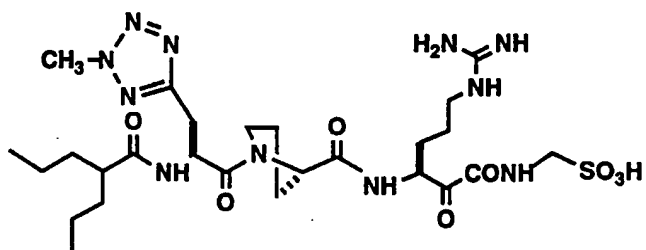
[8]



10 [9]



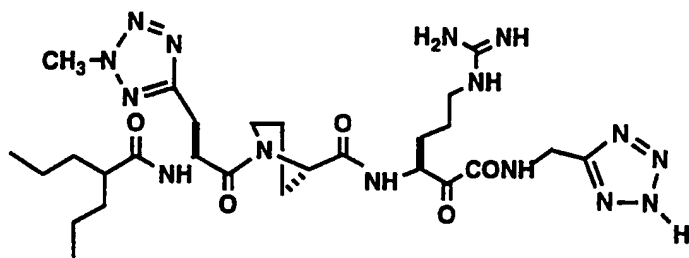
[10]



and

153

[11]



32. A compound of claim 1, wherein A_2 is $-(CH_2)_m-S(O_2)-R_2$.

5

33. A compound of claim 32, wherein R_2 is methyl.

34. A compound of claim 33, wherein m is 2.

10

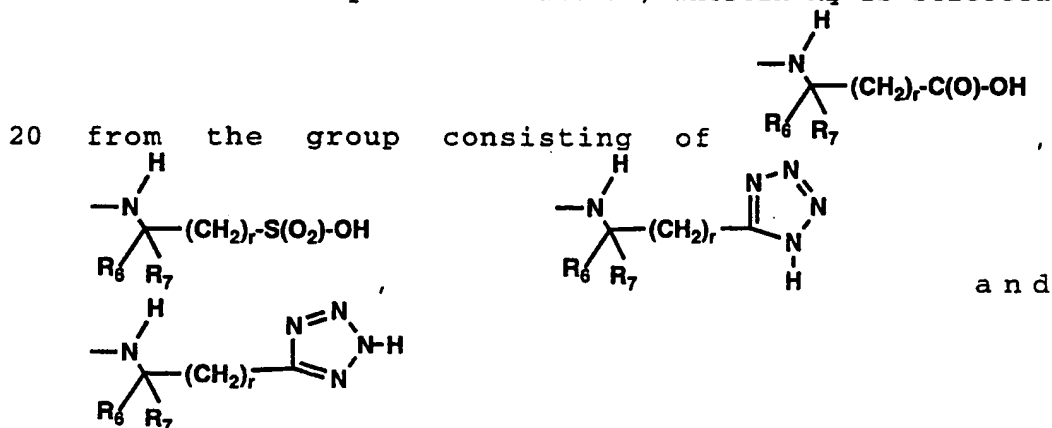
35. A compound of claim 34, wherein A_1 is $R_1-C(O)-$.

36. A compound of claim 35, wherein R_1 is selected from the group consisting of 2,2-dimethylpropyl, 3-methylbutyl and 1-propylbutyl.

15

37. A compound of claim 36, wherein A_3 is L-proline.

38. A compound of claim 37, wherein A_4 is selected

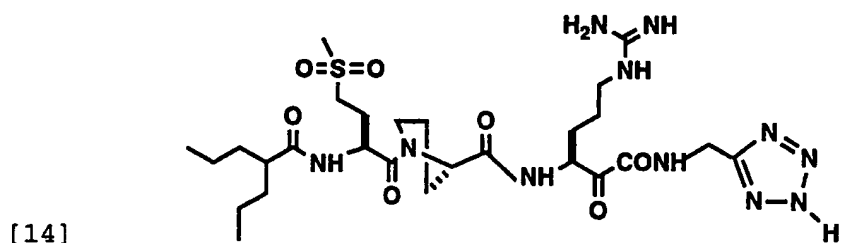
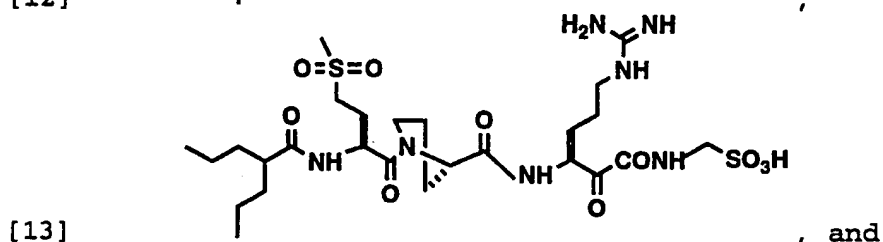
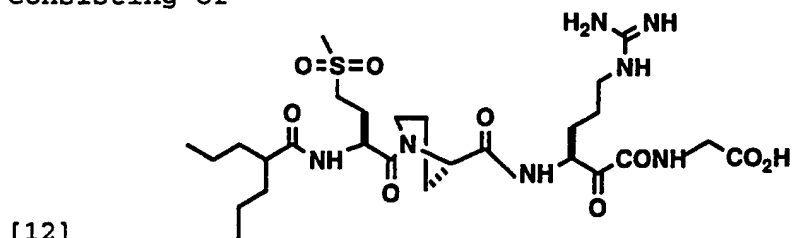


39. A compound of claim 38, wherein R_6 and R_7 are each hydrogen.

25

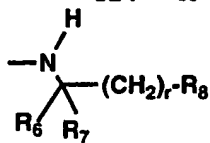
40. A compound of claim 39, wherein r is 0.

41. A compound of claim 32 selected from the group
5 consisting of



10

42. A compound of claim 37, wherein A4 is



43. A compound of claim 44, wherein R6 and R7 are
15 each hydrogen.

44. A compound of claim 43, wherein r is 1.

45. A compound of claim 44, wherein R8 is selected
20 from the group consisting of alkyl of 1 to about 12
carbon atoms, aryl of about 6 to about 14 carbon atoms

155

optionally mono-substituted with X_3 or optionally di-substituted with X_3 and X_4 , and aralkyl of about 6 to about 15 carbon atoms optionally mono-substituted with X_3 or optionally di-substituted with X_3 and X_4 .

5

46. A compound of claim 45, wherein R_8 is selected from the group consisting of $-\text{CH}(\text{CH}_3)_2$, phenyl, 4-methoxyphenyl and benzyl.

10

47. A compound of claim 34, wherein A_1 is selected from the group consisting of $R_1-\text{S}(\text{O}_2)-$ and $R_1-\text{NH}-\text{S}(\text{O}_2)-$.

48. A compound of claim 47, wherein A_1 is $R_1-\text{S}(\text{O}_2)-$.

15

49. A compound of claim 48, wherein R_1 is selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms and aralkyl of about 6 to about 15 carbon atoms.

20

50. A compound of claim 49, wherein R_1 is selected from the group consisting of 1-butyl, 2-naphthyl and benzyl.

25

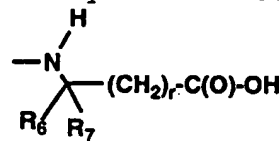
51. A compound of claim 50, wherein A_3 is selected from a group consisting of trans-4-hydroxy-L-proline, L-proline, L-leucine and L-isoleucine.

30

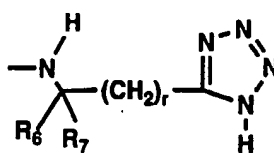
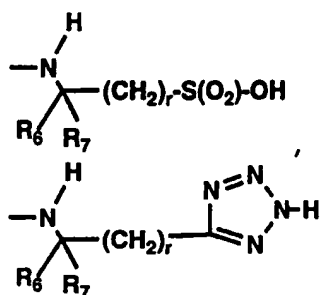
52. A compound of claim 51, wherein A_3 is L-proline.

53. A compound of claim 52, wherein A_4 is selected

from the group consisting of



156

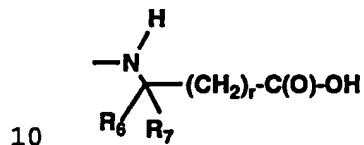


and

54. A compound of claim 53, wherein R₆ and R₇ are each hydrogen.

55. A compound of claim 54, wherein r is 0.

56. A compound of claim 53, wherein A₄ is



57. A compound of claim 56, wherein R₆ is hydrogen.

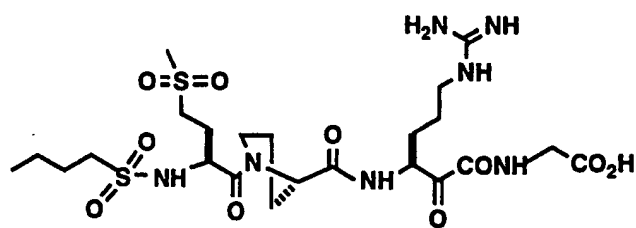
58. A compound of claim 57, wherein R₇ is selected from the group consisting of alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms and alkylene of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -OH, -C(O)-OH, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and -NH-S(O₂)-CH₃.

59. A compound of claim 58, wherein r is 0.

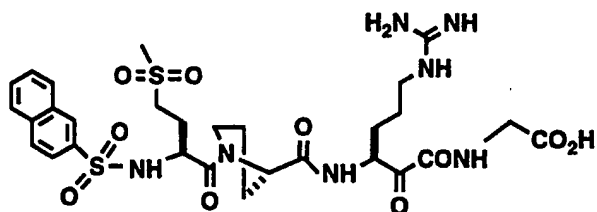
60. A compound of claim 32 selected from the group consisting of

157

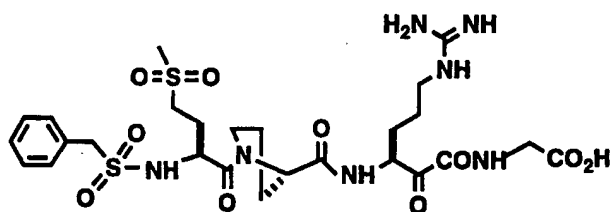
[15]



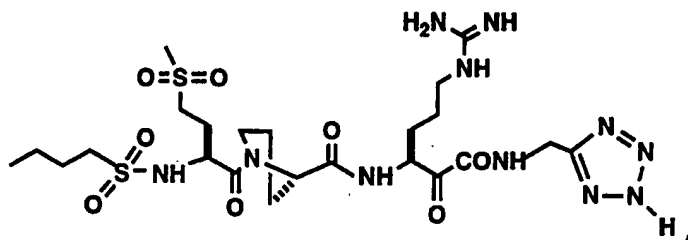
[16]



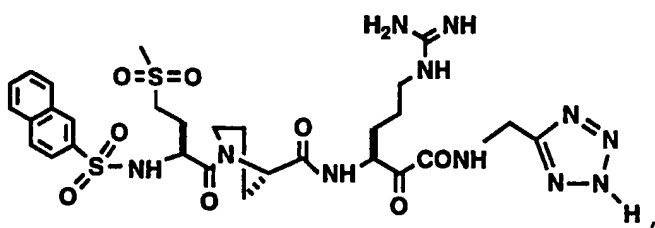
5 [17]



[18]

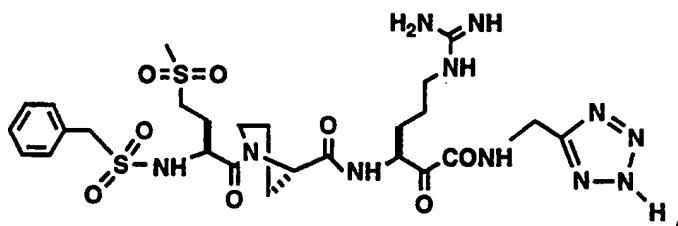


[19]



10

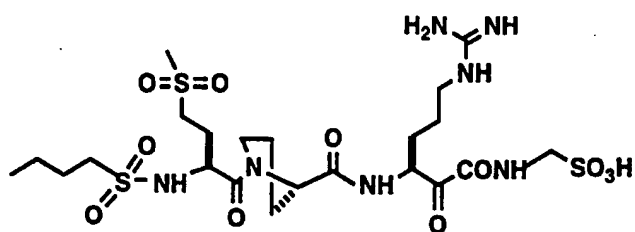
[20]



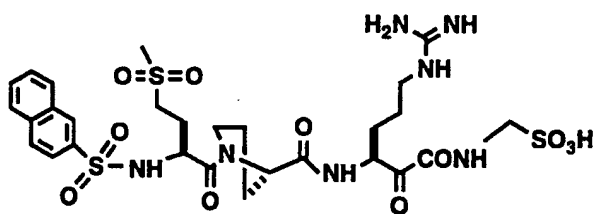
SUBSTITUTE SHEET

158

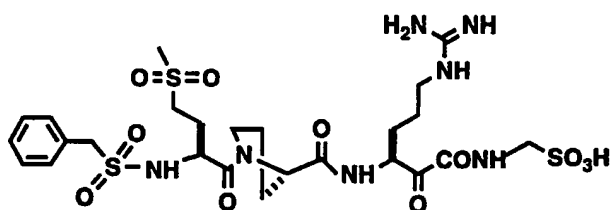
[21]



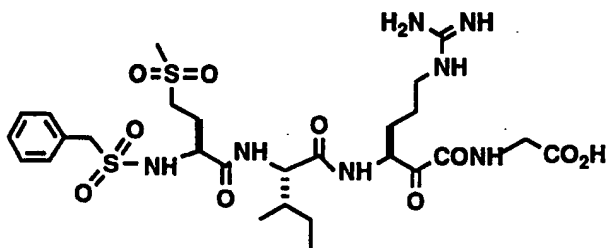
[22]



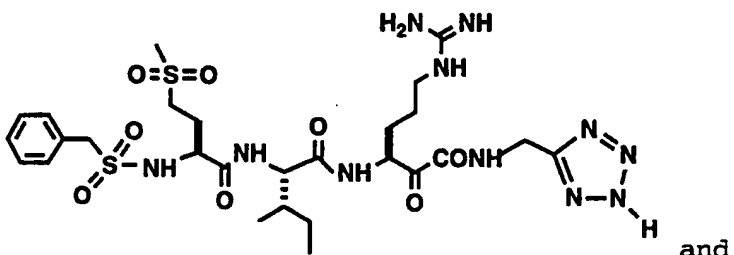
5 [23]



[24]



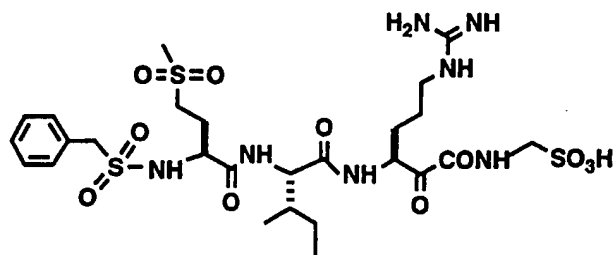
[25]



and

10

159



[26]

61. A compound of claim 1, wherein A₂ is hydrogen.

5 62. A compound of claim 61, wherein A₁ is selected from the group consisting of R₁-S(O₂)- and R₁-NH-S(O₂)-.

63. A compound of claim 62, wherein A₁ is R₁-S(O₂)-.

10

64. A compound of claim 63, wherein R₁ is selected from the group consisting of alkyl of 1 to about 12 carbon atoms, aryl of about 6 to about 14 carbon atoms and aralkyl of about 6 to about 15 carbon atoms.

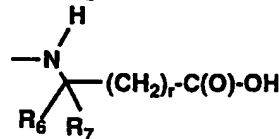
15

65. A compound of claim 64, wherein R₁ is selected from the group consisting of 1-butyl, 2-naphthyl and benzyl.

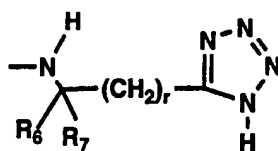
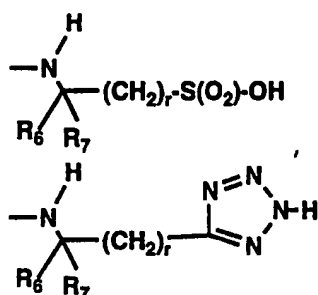
20 66. A compound of claim 65, wherein A₃ is L-proline.

67. A compound of claim 66, wherein A₄ is selected

from the group consisting of



160

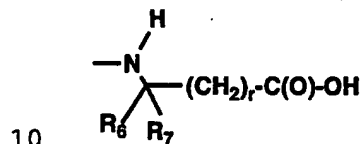


and

68. A compound of claim 67, wherein R₆ and R₇ are
5 each hydrogen.

69. A compound of claim 68, wherein r is 0.

70. A compound of claim 67, wherein A₄ is



10

71. A compound of claim 70, wherein R₆ is hydro-
gen.

15 72. A compound of claim 71, wherein R₇ is selected
from the group consisting of alkyl of 1 to about 4
carbon atoms, aryl of about 6 to about 14 carbon atoms,
aralkyl of about 6 to about 15 carbon atoms and alkylene
of 1 to about 4 carbon atoms substituted with a sub-
20 stituent selected from the group consisting of -OH,
-C(O)-OH, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and
-NH-S(O₂)-CH₃.

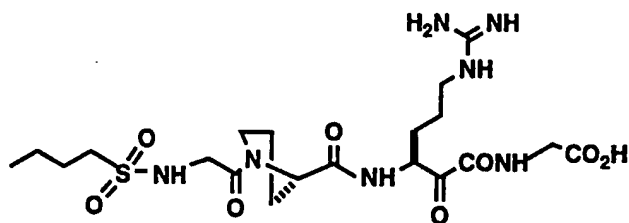
73. A compound of claim 72, wherein r is 0.

25

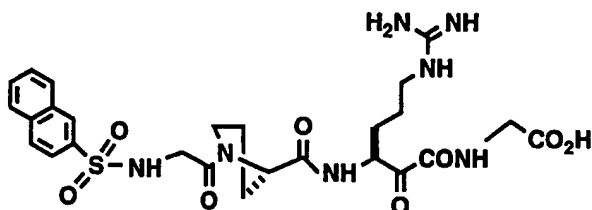
74. A compound of claim 61 selected from the group
consisting of

161

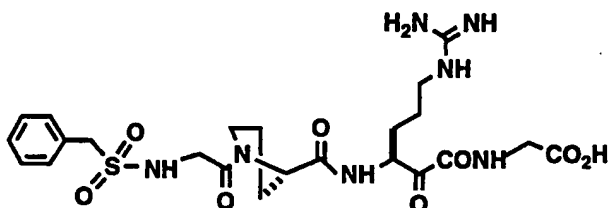
[27]



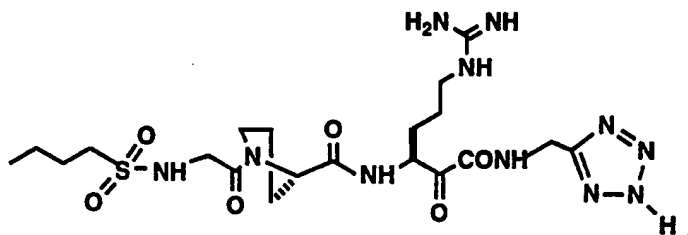
[28]



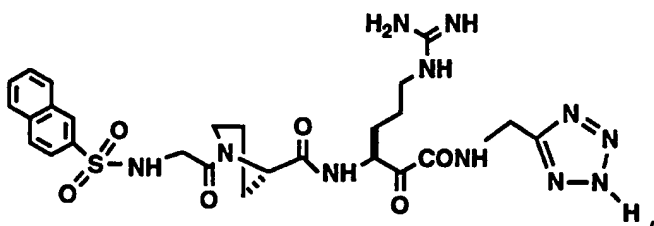
5 [29]



[30]

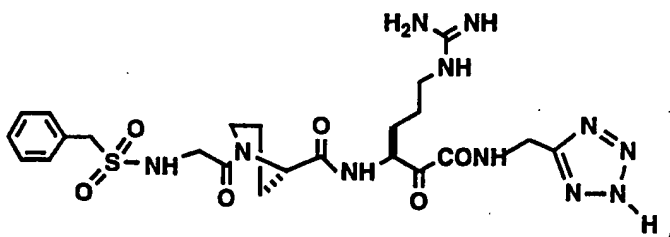


[31]



10

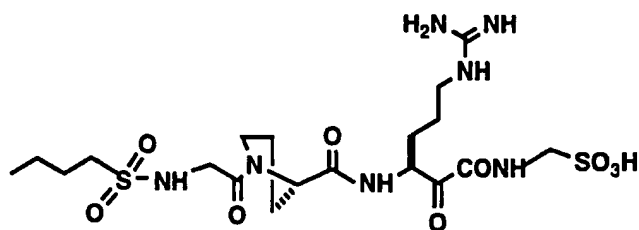
[32]



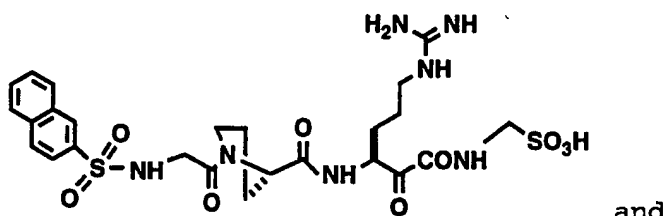
SUBSTITUTE SHEET

162

[33]

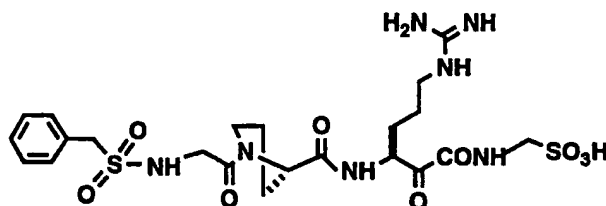


[34]

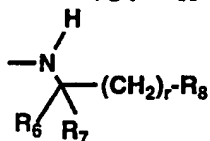


and

5 [35]



75. A compound of claim 66, wherein A4 is



10 76. A compound of claim 75, wherein R₆ and R₇ are each hydrogen.

77. A compound of claim 76, wherein r is 1.

15 78. A compound of claim 77, wherein R₈ is selected from the group consisting of alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄, and aralkyl of about 6 to
 20 about 15 carbon atoms optionally mono-substituted with X₃ or optionally di-substituted with X₃ and X₄.

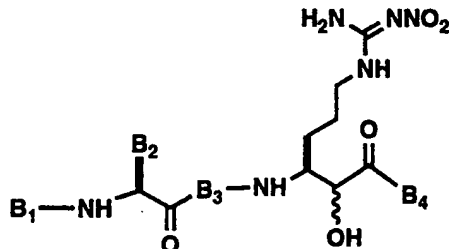
163

79. A compound of claim 78, wherein R₈ is selected from the group consisting of -CH(CH₃)₂, phenyl and benzyl.

80. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and therapeutically effective amount of the compound of claim 1.

81. A method of preventing or treating in a mammal a condition characterized by abnormal thrombus formation comprising administering to said mammal a therapeutically effective amount of the compound of claim 1, 2, 4, 12, 13, 20, 21, 22, 25, 30, 31, 32, 35, 40, 41, 46, 47, 55, 59, 60, 61, 62, 69, 73, 74 or 79.

82. A compound of the formula



wherein

(II)

(a) B₁ is selected from the group consisting of R₉-C(O)-, R₉-O-C(O)-, R₉-NH-C(O)-, R₉-S(O₂)-, R₉-O-S(O₂)- and R₉-NH-S(O₂)-, wherein R₉ is selected from the group consisting of

alkyl of 1 to about 12 carbon atoms,

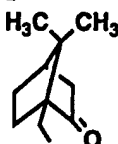
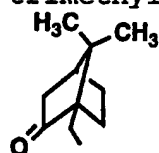
alkenyl of about 3 to about 6 carbon atoms,

aryl of about 6 to about 14 carbon atoms which is optionally mono-substituted with X₅ or optionally di-substituted with X₅ and X₆,

aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted in the aryl ring with X₅ or optionally di-substituted in the aryl ring with X₅ and X₆,

aralkenyl of about 8 to about 15 carbon atoms which is optionally mono-substituted in the aryl ring with X₅ or optionally di-substituted in the aryl ring with X₅ and X₆,

- 5 perfluoroalkyl of 1 to about 12 carbon atoms,
perfluoroaryl of about 6 to about 14 carbon atoms,
trimethylsilylalkyl of 4 to about 8 carbon atoms,

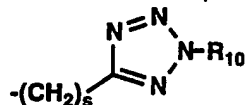
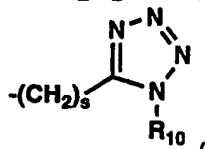


- , and , wherein X₅ and X₆ are independently selected from the group consisting of
10 bromo, chloro, fluoro, Y₃-, Y₃-O-, Y₃-O-C(O)-NH-, Y₃-O-C(O)-N(Y₄)-, (Y₃,Y₄)N-, Y₃-C(O)-NH-, Y₃-S-, Y₃-S(O)-, Y₃-S(O₂)-, Y₃-O-S(O₂)-, NH₂-S(O₂)- and Y₃-NH-S(O₂)-, wherein Y₃ and Y₄ are independently selected from the group consisting of trifluoromethyl, pentafluoroethyl,
15 aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms and alkyl of 1 carbon atom to about 12 which is optionally mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms;

- (b) B₂ is selected from the group consisting of
20 hydrogen,

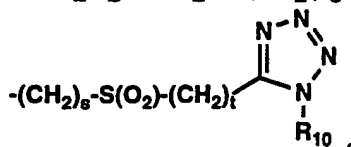
R₁₀-,

-(CH₂)_s-C(O)-O-R₁₀,

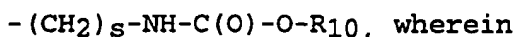
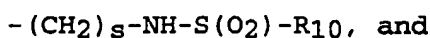
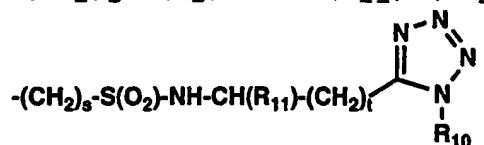
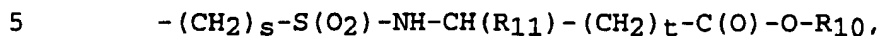
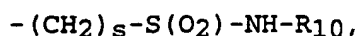
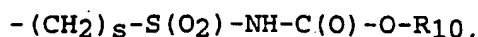
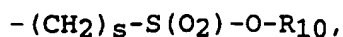
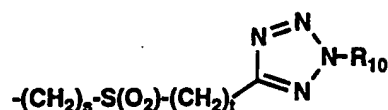


- 25 -(CH₂)_s-S(O₂)-R₁₀,

-(CH₂)_s-S(O₂)-(CH₂)_t-C(O)-O-R₁₀,



165



10 (i) s is 1, 2 or 3;

(ii) t is 0, 1, 2, 3 or 4;

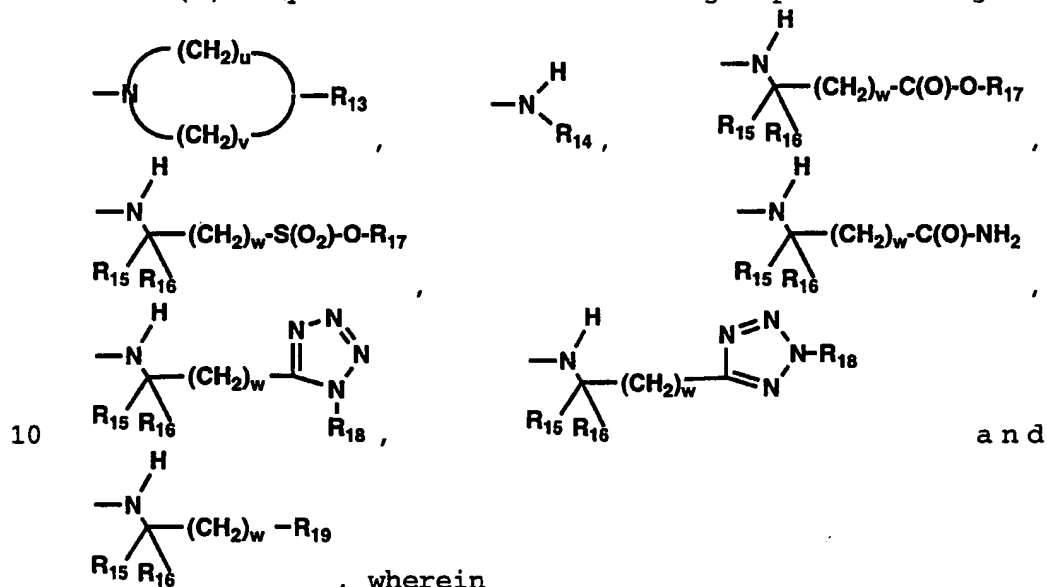
(iii) R_{10} is selected from the group consisting of alkenyl of about 3 to about 6 carbon atoms, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms, aralkenyl of about 8 to about 15 carbons atoms and alkyl of 1 to about 12 carbon atoms which is optionally mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms; and

(iv) R_{11} is selected from the group consisting of hydrogen, alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms, aralkyl of about 6 to about 15 carbon atoms, and alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of $-O-R_{10}$, $-C(O)-O-R_{10}$, $-C(O)-NH_2$, $-S-CH_3$, $-S(O)-CH_3$, $-S(O_2)-CH_3$ and $-NH-S(O_2)-CH_3$;

(c) B_3 is an amino acid residue of the amino acid selected from the group consisting of L-alanine, L-azetidinecarboxylic acid, glycine, L-isoleucine, L-leucine, L-lysine mono-substituted at its ϵ -amino group with $R_2-S(O_2)-$, L-methionine sulfone, N-methylglycine, L-ornithine mono-substituted at its δ -amino group with

R₂-S(O₂)-, L-pipecolic acid, L-phenylalanine, L-proline, L-valine, and trans-4-hydroxy-L-proline substituted at 4-hydroxy group with R₁₂-O-C(O)-, wherein R₁₂ is selected from the group consisting of alkyl of 1 to about 12 carbon atoms and aralkyl of about 6 to about 15 carbon atoms; and

(d) B₄ is selected from the group consisting of



- (i) u and v are each independently selected integers from 1 to 5 wherein the sum of u + v is 4 to 8;
- (ii) R₁₃ is aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, alkoxy of 1 to about 4 carbon atoms, -NH-C(O)-O-X₇, -C(O)-O-X₇, -C(O)-NH₂, fluoro, -O-X₇, -NO₂ and -CF₃;
- 15 (iii) R₁₄ is aryl of about 6 to about 14 carbon atoms;
- (iv) R₁₅ is selected from the group consisting of hydrogen and alkyl of 1 to about 4 carbon atoms;
- (v) R₁₆ is selected from the group consisting
- 20 of
- 25 hydrogen,
- alkyl of 1 to about 4 carbon atoms,

aryl of about 6 to about 14 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH-C(O)-O-X₈, -C(O)-O-X₈, -C(O)-NH₂, fluoro, -O-X₈, -NO₂,
5 -CF₃, alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms,

aralkyl of about 6 to about 15 carbon atoms which is optionally substituted with 1 or 2 substituents each independently selected from the group consisting of -NH-C(O)-O-X₉, -C(O)-O-X₉, -C(O)-NH₂, fluoro, -O-X₉, -NO₂,
10 -CF₃, alkyl of 1 to about 4 carbon atoms, and alkoxy of 1 to about 4 carbon atoms, and

alkyl of 1 to about 4 carbon atoms substituted with a substituent selected from the group consisting of -O-X₁₀, -C(O)-O-X₁₀, -C(O)-NH₂, -S-CH₃, -S(O)-CH₃, -S(O₂)-CH₃, and -NH-S(O₂)-CH₃;
15

(vi) R₁₇ is selected from the group consisting of alkyl of 1 to about 4 carbon atoms and aralkyl of about 6 to 15 carbon atoms;

20 (vii) R₁₈ is alkyl of 1 to about 12 carbon atoms which is optionally mono-substituted with aralkyloxy of about 6 to about 15 carbon atoms;

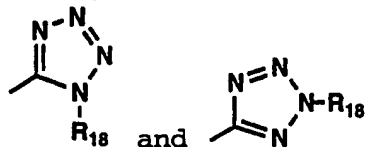
(viii) R₁₉ is selected from the group consisting of hydrogen atom, aryl of about 6 to about 14 carbon atoms which is optionally mono-substituted with X₁₁ or optionally di-substituted with X₁₁ and X₁₂; and aralkyl of about 6 to about 15 carbon atoms which is optionally mono-substituted with X₁₁ or optionally di-substituted with X₁₁ and X₁₂; and
25

30 (ix) w is 0, 1, 2, 3, 4 or 5;

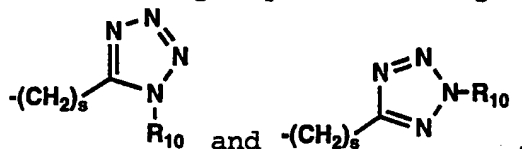
wherein X₇, X₈, X₉ and X₁₀ are independently selected from the group consisting of alkyl of 1 to about 4 carbon atoms, aryl of about 6 to about 14 carbon atoms and aralkyl of about 6 to about 15 carbon atoms;
35 and

168

wherein X₁₁ and X₁₂ are independently selected from the group consisting of -C(O)-O-R₁₇, -S(O₂)-O-R₁₇,



- 5 83. A compound of claim 82, wherein B₂ is selected from the group consisting of -(CH₂)_s-C(O)-O-R₁₀,



84. A compound of claim 83, wherein R₁₀ is
10 selected from the group consisting of benzyl and benzyloxymethyl.

85. A compound of claim 84, wherein s is 1 or 2.

- 15 86. A compound of claim 85, wherein B₁ is selected from the group consisting of R₉-C(O).

87. A compound of claim 86, wherein R₉ is selected from the group consisting of 2,2-dimethylpropyl, 3-methylbutyl and 1-propylbutyl.
20

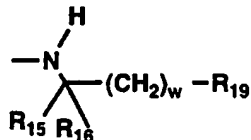
88. A compound of claim 85, wherein B₁ is R₉-O-C(O)-.

- 25 89. A compound of claim 88, wherein R₉ is 1,1-dimethylethyl.

90. A compound of claim 87 or 89, wherein B₃ is L-proline.
30

169

91. A compound of claim 90, wherein B₄ is



92. A compound of claim 91, wherein R₁₅ and R₁₆
5 are each hydrogen.

93. A compound of claim 92, wherein w is 1.

94. A compound of claim 93, wherein R₁₉ is
10 selected from the group consisting of -CH(CH₃)₂, phenyl
and benzyl.

95. A compound of claim 83, wherein R₁₀ is alkyl
of 1 to about 12 carbon atoms.
15

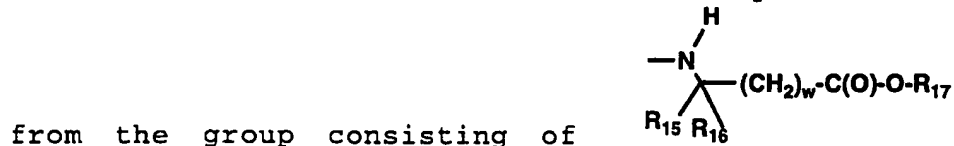
96. A compound of claim 95, wherein R₁₀ is methyl.

97. A compound of claim 96, wherein B₁ is R₉-C(O)-.

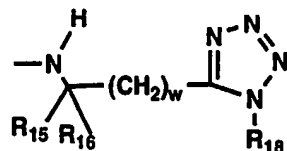
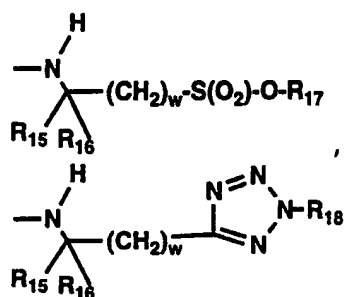
98. A compound of claim 97, wherein R₉ is selected
20 from the group consisting of 2,2-dimethylpropyl, 3-
methylbutyl and 1-propylbutyl.

99. A compound of claim 98, wherein B₃ is L-
25 proline.

100. A compound of claim 99, wherein B₄ is selected



170



and

101. A compound of claim 100, wherein R₁₅ and R₁₆
5 are each hydrogen.

102. A compound of claim 101, wherein w is 0.

103. A compound of claim 102, wherein R₁₇ is benzyl
10 and R₁₈ is benzyloxymethyl.

104. A compound of claim 82, wherein B₂ is
-(CH₂)_s-S(O₂)-R₁₀.

105. A compound of claim 104, wherein R₁₀ is
15 methyl.

106. A compound of claim 105, wherein s is 2.

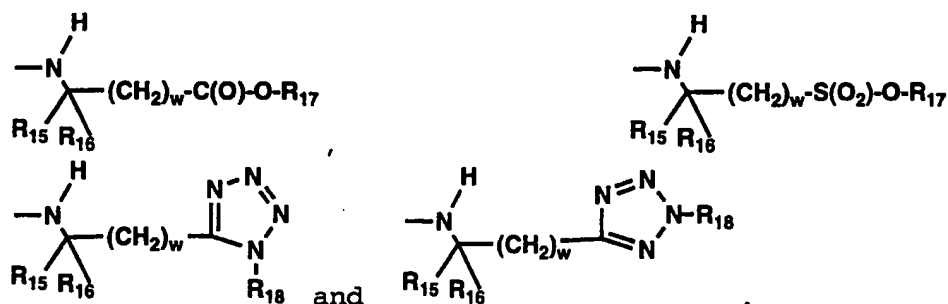
107. A compound of claim 106, wherein B₁ is R₉-
20 C(O)-.

108. A compound of claim 107, wherein R₉ is
selected from the group consisting of 2,2-dimethyl-
25 propyl, 3-methylbutyl and 1-propylbutyl.

109. A compound of claim 108, wherein B₃ is L-
proline.

110. A compound of claim 109, wherein B₄ is
selected from the group consisting of

171

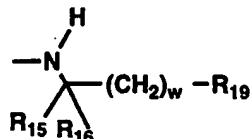


111. A compound of claim 110, wherein R₁₅ and R₁₆ are each hydrogen.

112. A compound of claim 111, wherein w is 0.

113. A compound of claim 112, wherein R₁₇ is benzyl and R₁₈ is benzyloxymethyl.

114. A compound of claim 109, wherein B₄ is



115. A compound of claim 114, wherein R₁₅ and R₁₆ are each hydrogen.

116. A compound of claim 115, wherein w is 1.

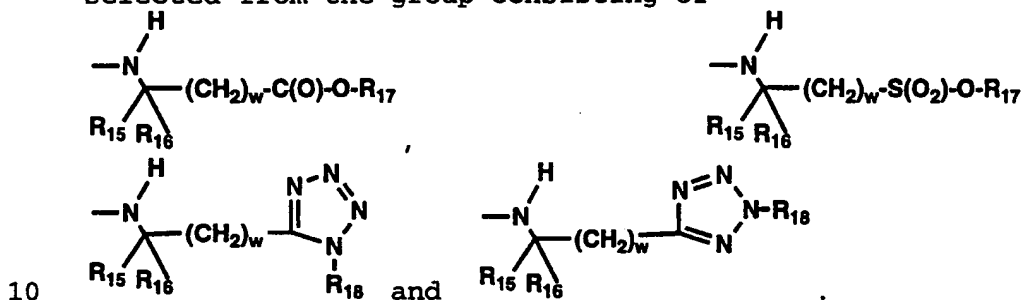
117. A compound of claim 116, wherein R₁₉ is selected from the group consisting of -CH(CH₃)₂, 4-methoxyphenyl, phenyl and benzyl.

118. A compound of claim 106, wherein B₁ is R₉-S(O₂)-.

119. A compound of claim 118, wherein R₉ is selected from the group consisting of 1-butyl, 2-naphthyl and benzyl.

120. A compound of claim 119, wherein B₃ is selected from the group consisting of L-proline, L-leucine, L-isoleucine and trans-4-hydroxy-L-proline substituted at 4-hydroxy group with benzyloxycarbonyl.

121. A compound of claim 120, wherein B₄ is selected from the group consisting of



122. A compound of claim 121, wherein R₁₅ and R₁₆ are each hydrogen.

15 123. A compound of claim 122, wherein w is 0.

124. A compound of claim 123, wherein R₁₇ is benzyl and R₁₈ is benzyloxymethyl.

20 125. A compound of claim 82, wherein B₂ is hydrogen.

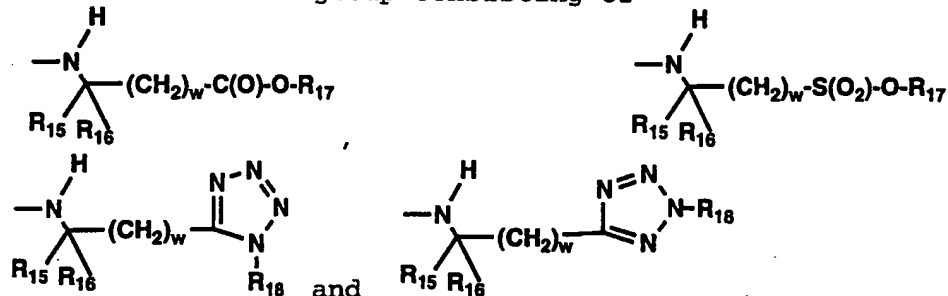
25 126. A compound of claim 125, wherein B₁ is R₉-S(=O)₂-.

127. A compound of claim 126, wherein R₉ is selected from the group consisting of 1-butyl, 2-naphthyl and benzyl.

30 128. A compound of claim 127, wherein B₃ is L-proline.

173

129. A compound of claim 128, wherein B₄ is selected from the group consisting of



5

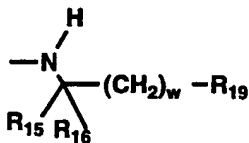
130. A compound of claim 129, wherein R₁₅ and R₁₆ are each hydrogen.

131. A compound of claim 130, wherein w is 0.

10

132. A compound of claim 131, wherein R₁₇ is benzyl and R₁₈ is benzyloxymethyl.

133. A compound of claim 128, wherein B₄ is



15

134. A compound of claim 133, wherein R₁₅ and R₁₆ are each hydrogen.

20

135. A compound of claim 134, wherein w is 1.

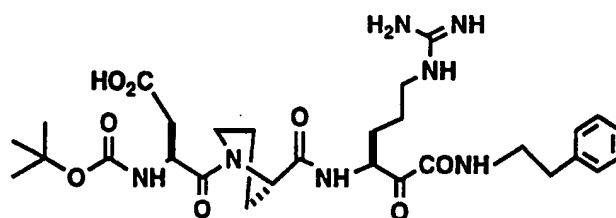
136. A compound of claim 135, wherein R₁₉ is selected from the group consisting of -CH(CH₃)₂, phenyl and benzyl.

25

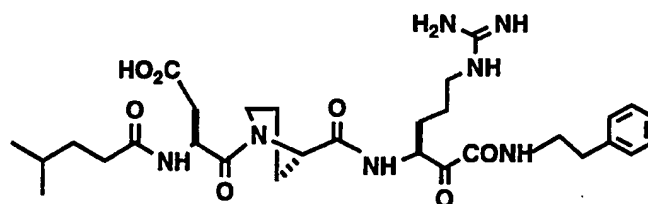
137. A compound of claim 1, selected from the group consisting of:

174

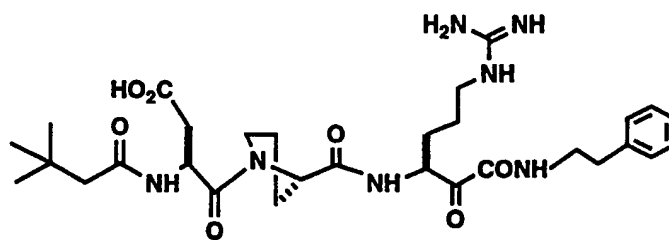
[1]



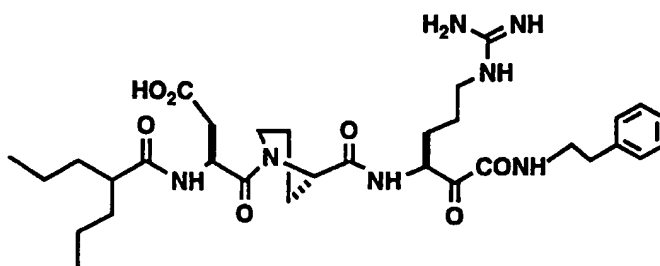
[2]



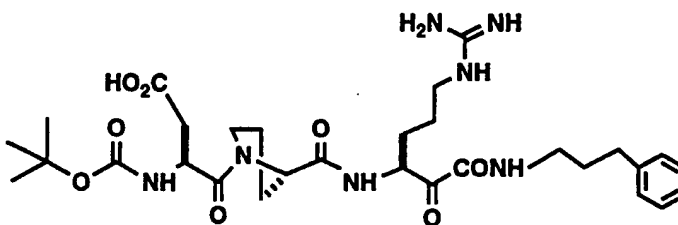
5 [3]



[4]

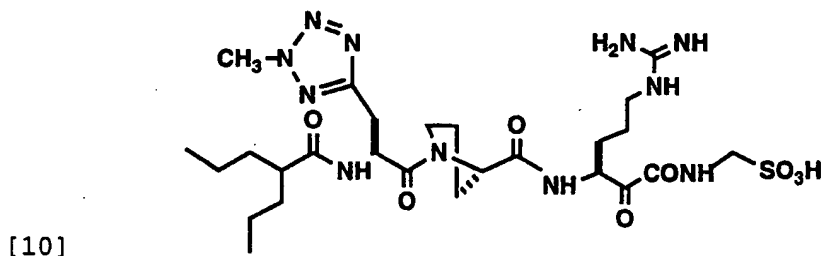
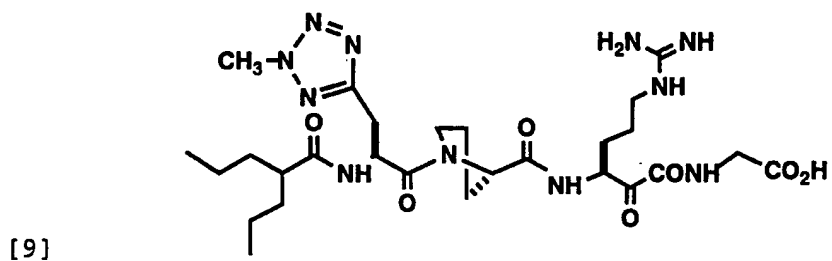
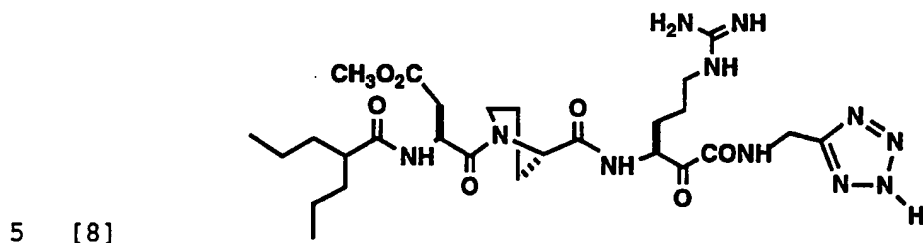
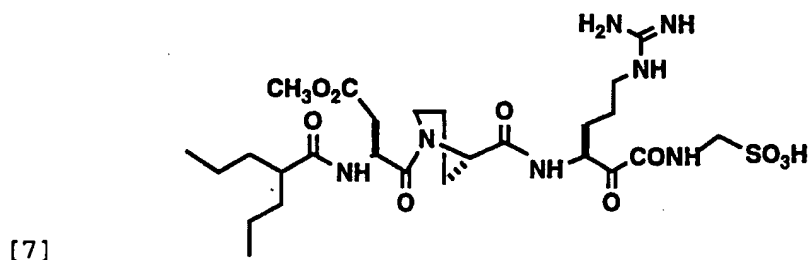
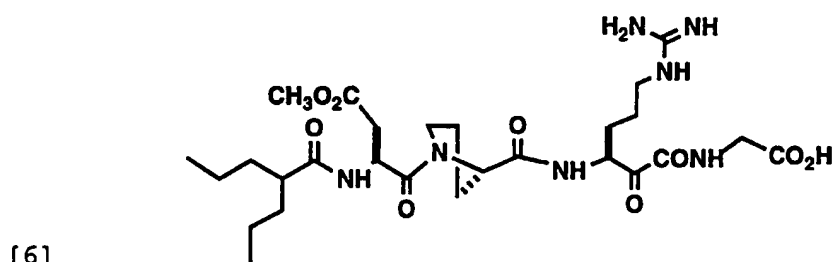


[5]



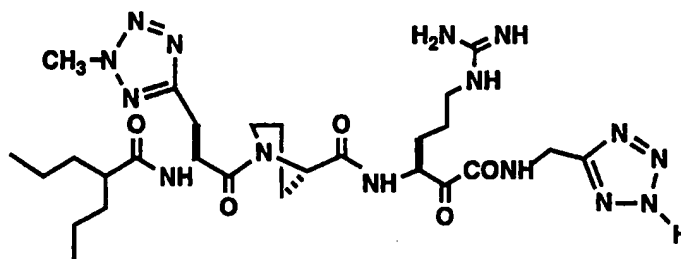
10

175

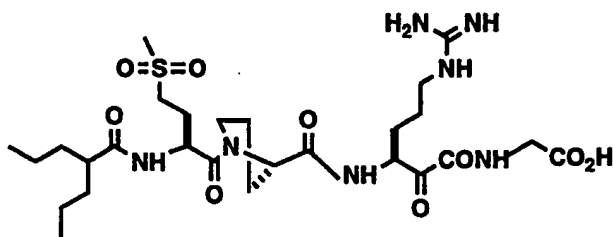


176

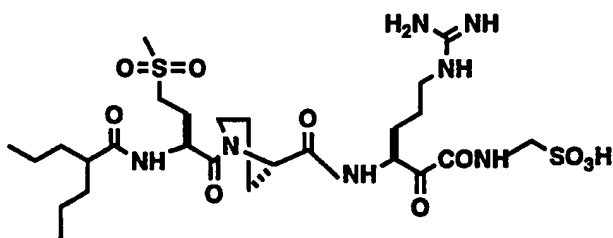
[11]



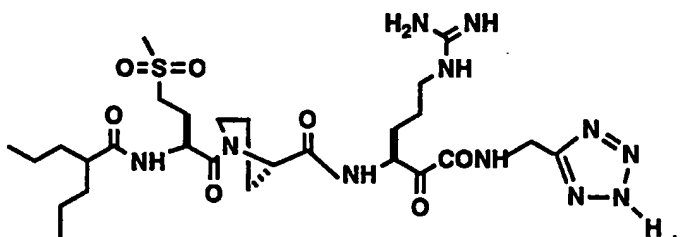
[12]



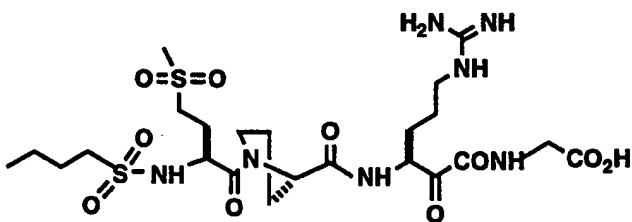
5 [13]



[14]

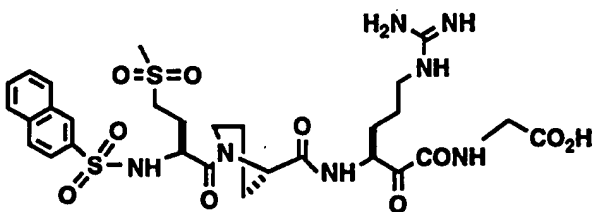


[15]



10

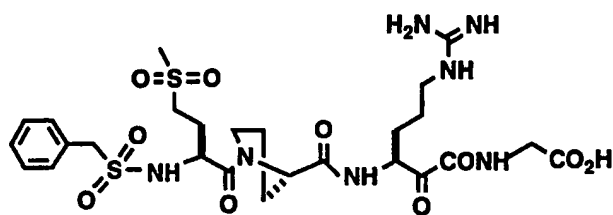
[16]



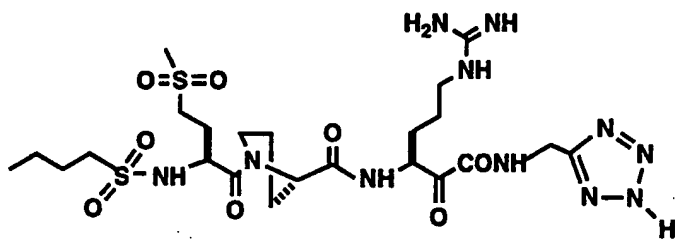
SUBSTITUTE SHEET

177

[17]

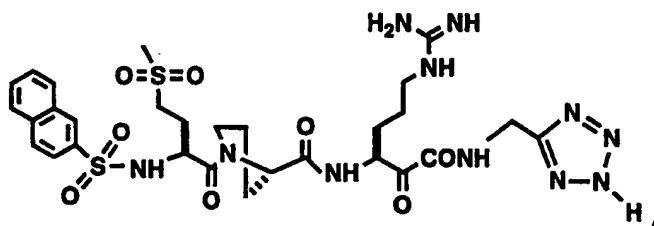


[18]

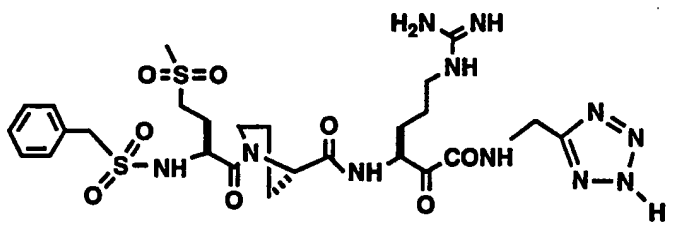


5

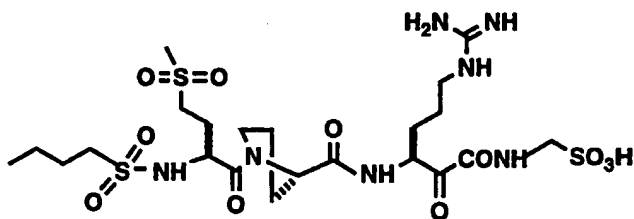
[19]



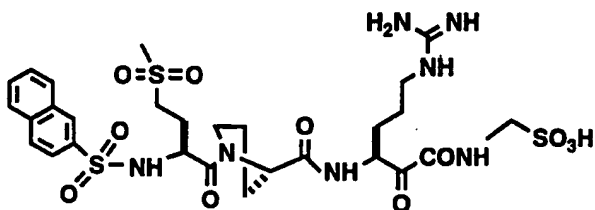
[20]



10 [21]



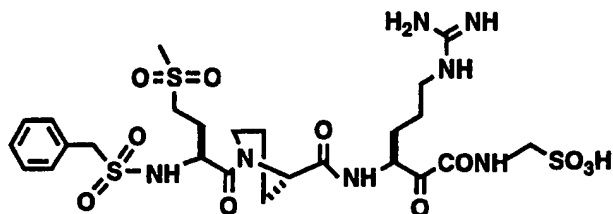
[22]



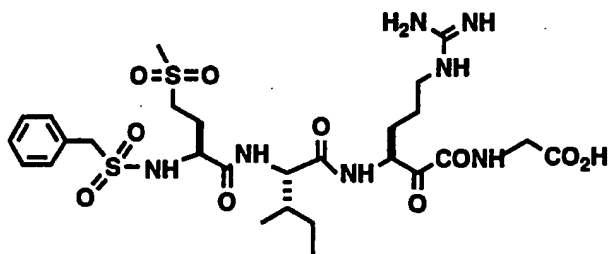
SUBSTITUTE SHEET

178

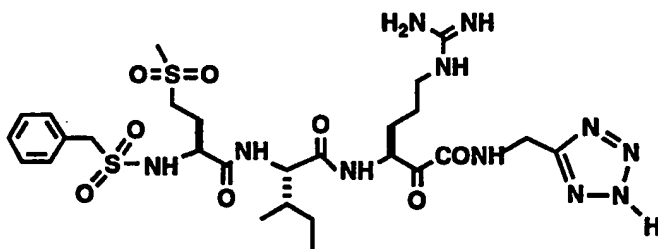
[23]



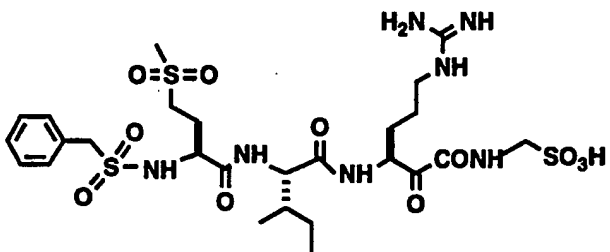
[24]



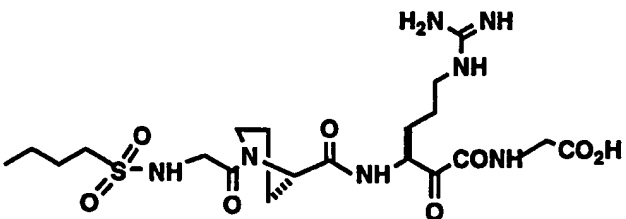
5 [25]



[26]



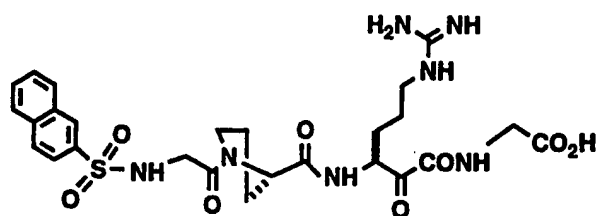
[27]



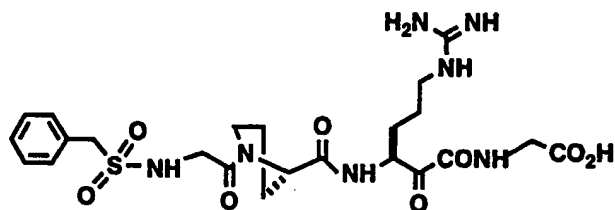
10

179

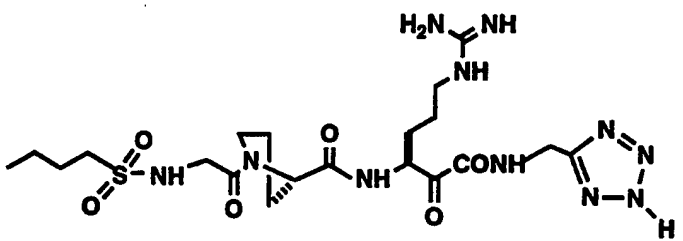
[28]



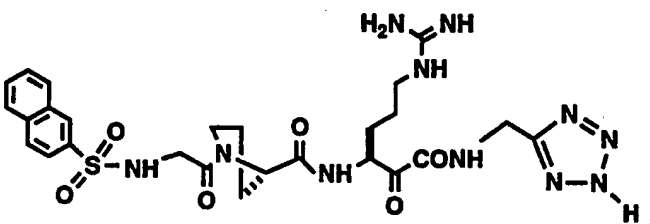
[29]



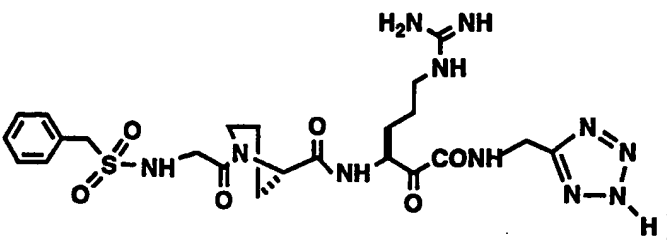
5 [30]



[31]

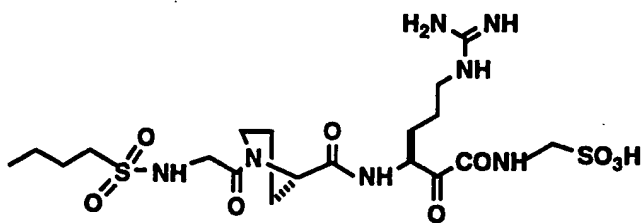


[32]



10

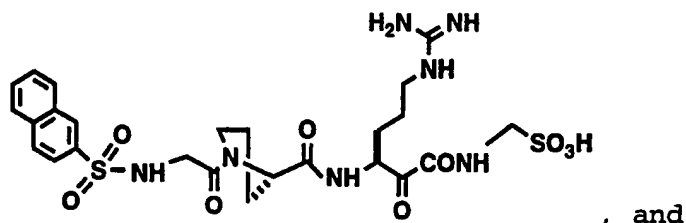
[33]



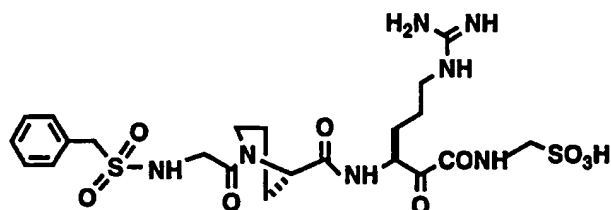
SUBSTITUTE SHEET

180

[34]



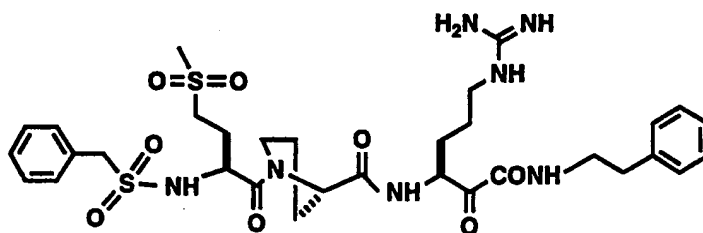
[35]



5

138. A compound of claim 1 having the formula:

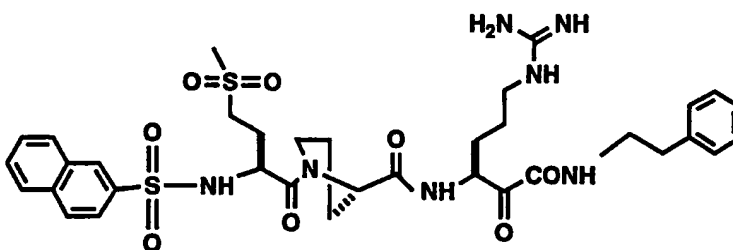
[36]



10

139. A compound of claim 1 having the formula:

[37]

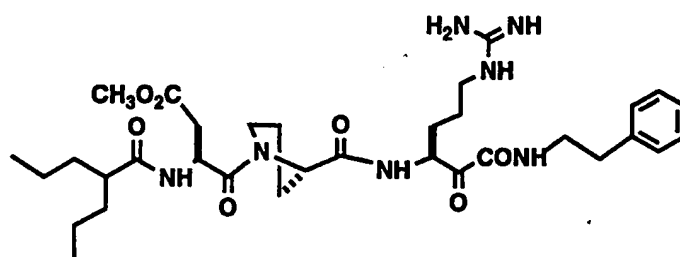


15

140. A compound of claim 1 having the formula:

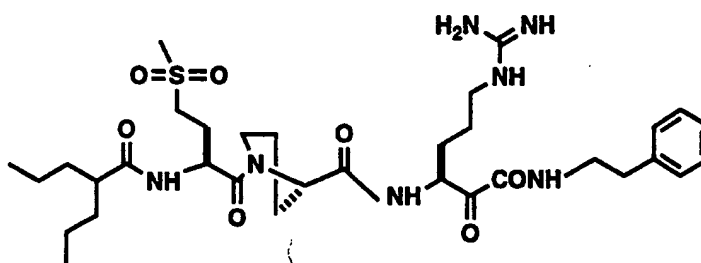
181

[38]



141. A compound of claim 1 having the formula:

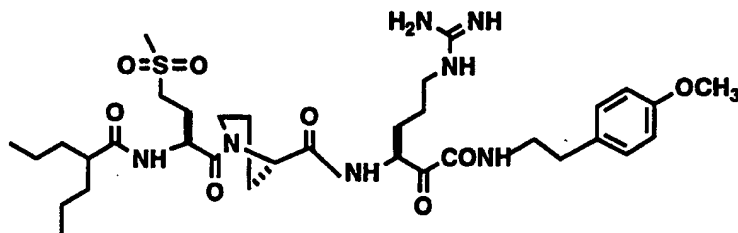
5 [39]



142. A compound of claim 1 having the formula:

[40]

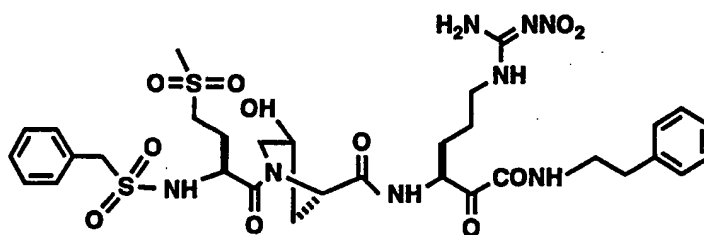
10



143. A compound of claim 1 having the formula:

[41]

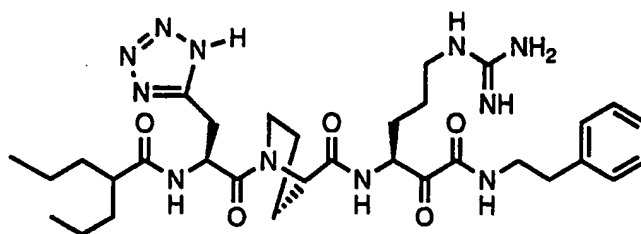
15



144. A compound of claim 1 having the formula:

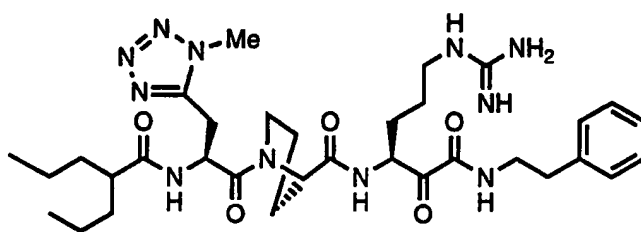
182

[42]



145. A compound of claim 1 having the formula:

5 [43]



1 / 1

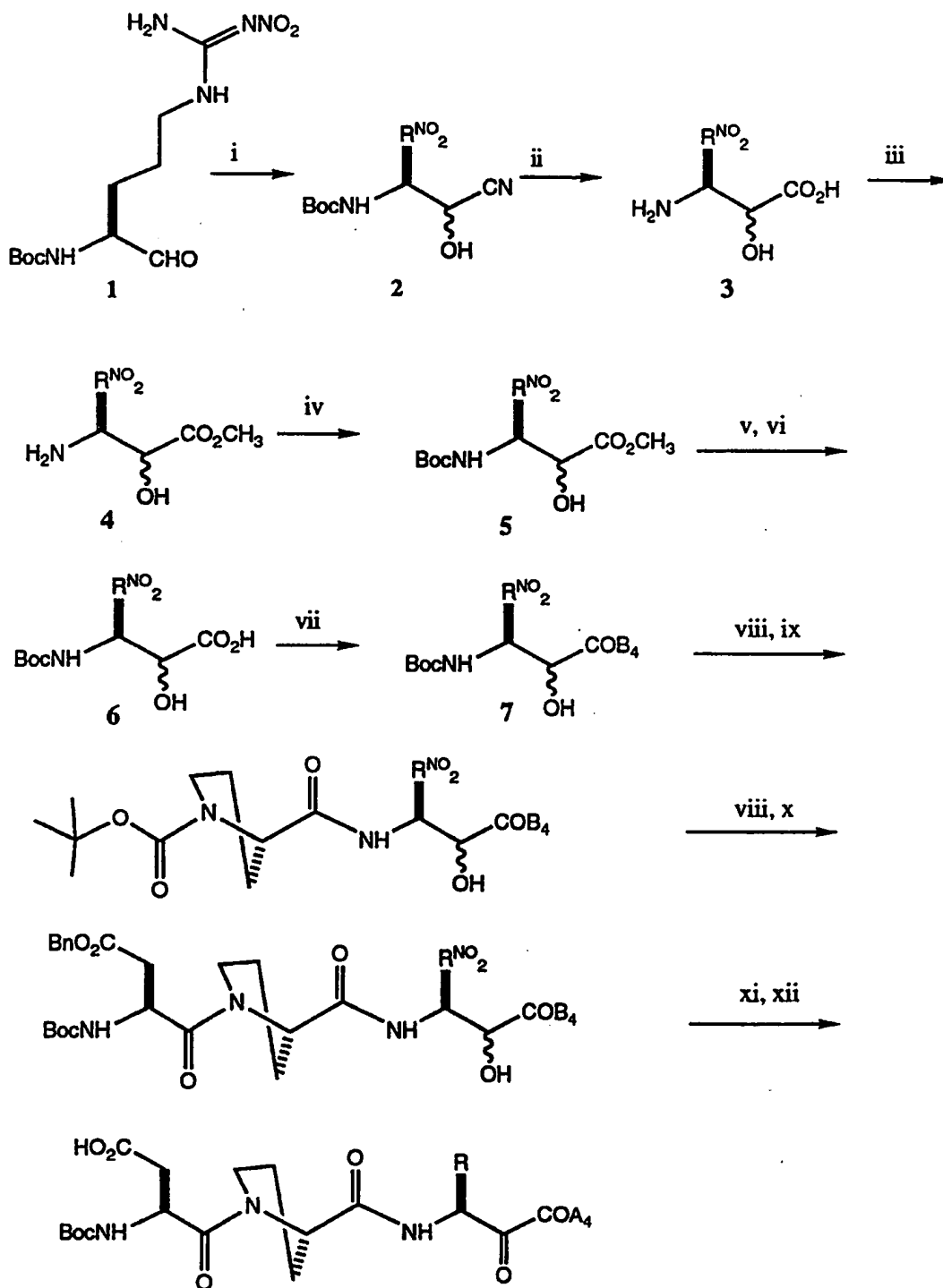


FIG. 1

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10015

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07C 69/76, 303/00, 233/00; A61K 37/00
US CL : 560/62; 564/80, 123; 514/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/18, 822; 530/331; 560/62; 564/80, 123

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, REG, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 3,966,701 (DORMAN ET AL.) 29 JUNE 1976, (see especially, column 1, lines 26-49).	1-145
Y	US, A, 4,478,745 (BAJUSZ ET AL.) 23 OCTOBER 1984, (see especially, column 3, lines 19-29).	1-145
Y,P	US, A, 5,221,752 (SOMENO ET AL.) 22 JUNE 1993, (see especially, column 1, lines 15-23 and claim 3; column 3, line 28 to column 4, line 5; column 7, line 40 to column 10, line 54; and column 10, lines 43-54).	1-145

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 FEBRUARY 1994

Date of mailing of the international search report

FEB 14 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

JON P. WEBER, PH.D.

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORTInter. application No.
PCT/US93/10015**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,171,299 (HAMBURGER ET AL.) 16 OCTOBER 1979, (see especially, column 4, line 48 to column 5, line 2 and column 7, line 35 to column 8, line 5).	1-145
A	US, A, 4,161,522 (HAMBURGER ET AL.) 17 JULY 1979, (see entire document).	1-145

INTERNATIONAL SEARCH REPORT

Inter. application No.

PCT/US93/10015

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(Form PCT/ISA/206 Previously Mailed.)

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☒

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10015

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This International Searching Authority has found 12 inventions claimed in the International Application covered by the claims indicated below:

To simplify the grouping of claims, X_n will refer to both A_n and B_n . Examiner notes that claim 13 is identical to claim 4. Further, examiner is assuming that claim 43 is meant to depend from claim 42 and not claim 44 as written. Examiner would appreciate applicants' carefully checking claims for consistency with the groupings.

I. Claims 1, 61, 80-82, 125 and 137, drawn to first compounds, compositions and method of use wherein $X_1 = R-CO-$, $X_2 = H$, $X_4 =$ non-heterocyclic, classified, for example, in Class 560, subclass 32, Class 564, subclasses 80 and 123 and Class 514, subclass 18.

II. Claims 1, 61, 80-82, 125 and 137, drawn to second compounds, compositions and method of use wherein $X_1 = R-CO-$, $X_2 = H$, $X_4 =$ heterocyclic, classified, for example, in Class 548, subclass 250, Class 560, subclass 32 and Class 514, subclass 18.

III. Claims 1-46, 80-117, 137 and 140-142, drawn to third compounds, compositions and method of use wherein $X_1 = R-CO-$, $X_2 =$ non-heterocyclic, $X_4 =$ non-heterocyclic, classified, for example, in Class 560, subclass 32, Classes 564, subclasses 80 and 132 and Class 514, subclass 18.

IV. Claims 1-7, 13-15, 22-30, 32-41, 80-90, 95-113 and 137, drawn to fourth compounds, compositions and method of use wherein $X_1 = R-CO-$, $X_2 =$ non-heterocyclic, $X_4 =$ heterocyclic, classified, for example, in Class 548, subclass 250, Class 560, subclass 32 and Class 514, subclass 18.

V. Claims 1-20, 22-40, 42-46, 80-103, 137 and 144-145, drawn to fifth compounds, compositions and method of use wherein $X_1 = R-CO-$, $X_2 =$ heterocyclic, $X_4 =$ non-heterocyclic, classified, for example, in Class 548, subclasses 400 and 579, Class 560, subclass 32.

VI. Claims 1-7, 13-15, 22-30, 32-40, 80-90, 95-103 and 137, drawn to sixth compounds, compositions and method of use wherein $X_1 = R-CO-$, $X_2 =$ heterocyclic, $X_4 =$ heterocyclic, classified, for example, in Class 548, subclasses 250, 400 and 579, Class 560, subclass 32.

VII. Claims 1, 61-82 and 125-137, drawn to seventh compounds, compositions and method of use wherein $X_1 = R-SO_2-$, $X_2 = H$, $X_4 =$ non-heterocyclic, classified, for example, in Class 558, subclass 44 and Class 562, subclass 125.

VIII. Claims 1, 61-69, 74, 80-82, 125-132 and 137, drawn to eighth compounds, compositions and method of use wherein $X_1 = R-SO_2-$, $X_2 = H$, $X_4 =$ heterocyclic, classified, for example, in Class 558, subclasses 44, 250, 400 and 579, Class 562, subclass 125, and Class 564, subclasses 80 and 123.

IX. Claims 1-3, 22-24, 32-33, 47-60, 80-85, 95-96, 104-106, 118-124, 137-139 and 143, drawn to ninth compounds, compositions and method of use wherein $X_1 = R-SO_2-$, $X_2 =$ non-heterocyclic, $X_4 =$ non-heterocyclic, classified, for example, in Class 558, subclass 44 and Class 562, subclass 125.

X. Claims 1-3, 22-24, 32-33, 47-55, 60, 80-85, 95-96, 104-106, 118-124 and 137, drawn to tenth compounds, compositions and method of use wherein $X_1 = R-SO_2-$, $X_2 =$ non-heterocyclic, $X_4 =$ heterocyclic, classified, for example, in Class 548, subclass 250, Class 558, subclass 44.

XI. Claims 1-3, 22-24, 32-33, 80-85, 95-96 and 137, drawn to eleventh compounds, compositions and method of use wherein $X_1 = R-SO_2-$, $X_2 =$ heterocyclic, $X_4 =$ non-heterocyclic, classified, for example, in Class 548, subclasses 400 and 579, Class 558, class 32.

XII. Claims 1-3, 22-24, 32-33, 80-85, 95-96 and 137, drawn to twelfth compounds, compositions and method of use wherein $X_1 = R-SO_2-$, $X_2 =$ heterocyclic, $X_4 =$ heterocyclic, classified, for example, in Class 548, subclasses 250, 400 and 579, Class 558, subclass 44 and Class 562, subclass 125.

International Searching Authority considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions listed as Groups I-XII do not meet the requirements for Unity of Invention for the following reasons: the different inventions groups do not share a common structural form. Examiner has indicated the differences in basic form as $R-CO-$ or $R-SO_2-$ at X_1 , hydrogen, non-heterocyclic or heterocyclic at X_2 , and non-heterocyclic or heterocyclic at X_4 . Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. Note that PCT Rule 13 does not provide for multiple products or methods within a single application.

Claims 1-20, 22-55, 60-69, 74, 80-113, 118-132 and 137 are generic to two or more Groups and will be searched insofar as the claims are encompassed by the Group definition for Groups for which the fees have been paid.